OCCURRENCE OF OCTADECATRIENOIC ACID ISOMERS IN APHIDS AND OTHER INSECTS

By

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CHAPTER I

INTRODUCTION

Aphids, as a group, are extremely successful and occur throughout the world with the greatest number of species in the temperate regions. Many are agricultural pests and can severely retard host plant growth. Several generations are produced each year and their complex life cycles and polymorphism enable them to exploit their hosts as well as respond to almost every contingency of their environment (Dixon, 1973).

Research on the relationship of aphids and their respective host plant(s) has been an area of on-going study for many years. By learning more about the biochemistry of these relationships, we may be able to find a way of controlling these pests without the use of insecticides harmful to the environment.

Lipids, particularly fatty acids, are essential to all organisms as components of membranes as well as other biochemical processes. While analyzing the fatty acid content of aphids by gas chromatography, an unusual isomer of octadecatrienoic acid was found that did not coelute with either 9,12,15-octadecatrienoic (α-linolenic) or the 6,9,12-octadecatrienoic (γ-linolenic acid). This isomer was isolated and purified from the pea aphid, <u>Acyrthosiphon</u> <u>pisum</u> (Harris). The structure was determined to be 9,12,17-octadecatrienoic acid by a combination of techniques including gas chromatography-mass spectrometry, ozonolysis, infrared spectroscropy, and proton- and ^{13}C-nuclear magnetic resonance (Dillwith, J. W., unpublished data). This isomer of linolenic acid was designated as β-linolenic acid (Fig. 1). A fourth isomer has also been partially characterized from aphids. This isomer which has been designated as δ-linolenic acid is similar to the β isomer, however, the double bond distribution has not yet been determined. The distribution of the common α and γ isomers in insects has not been adequately addressed in the literature and no information is available on the distribution of the unusual β and δ isomers.

The objectives of this study were to determine the distribution and occurrence of linolenic acid isomers 1) in selected aphids and other insects, 2) to determine the occurrence of linolenic acid isomers in neutral and polar lipids and 3) to record the fatty acid composition of all insects studied.

$\Delta^{6,9,12}$ –18 : 3 γ

$\Delta^{9,12,15}$–18 : 3 α

$\Delta^{9,12,17}$–18 : 3 β

? ? δ

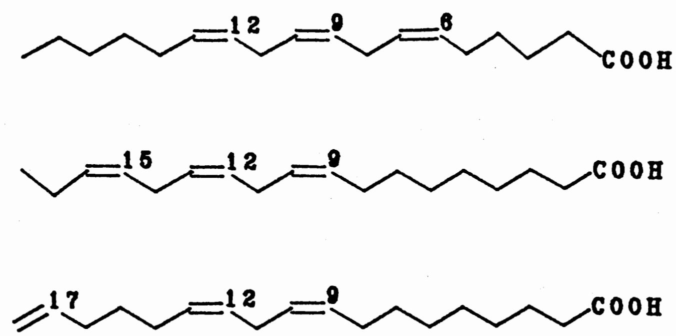


Figure 1. Isomers of Octadecatrienoic Acid

CHAPTER II

LITERATURE REVIEW

Lipids

Lipids are more soluble in organic than in aqueous media (Stryer, 1975; Gilbert, 1967). Their solubility depends on the amount and kind of polar functional groups. Lipids have relatively little capacity for hydrogen bonding or dipole-dipole interactions and a large capacity for van der Waals' interaction. Lipids are recognized as molecules in which the proportion of carbon atoms that possess no polar functionality is large. The molecules may be acyclic, linear, branched, mono- or polycyclic, and what polar functions there are may be quite varied. The low degree of polarity inherent in lipids is the key to their biological function. The nonpolar parts of the molecules, such as the hydrophobic entities, frequently exert their biological significance in a nonmetabolic way, as in the membranous role of fatty acids and sterols (Nes and Nes, 1980).

Lipids make up a large group of structurally heterogeneous compounds. These compounds are of great importance in insects as they have important roles in physiological processes such as reproduction, embryogenesis, and metamorphosis (Downer, 1985). Lipids are also used as pheromones and hormones, protection against desiccation, cell membranes and as a major source of energy (Downer and Matthews, 1976). The dominant lipid class in insects is triacylglycerol which is deposited in the fat body and serves as a reserve of metabolic energy. This energy can be mobilized and utilized for flight, during diapause and other non-feeding developmental life stages.

The Class Insecta contains more species than any other group of organisms. During their extensive evolutionary history, they developed many diverse morphological, physiological, and biochemical traits. Lipids have played a central role of ensuring the success of the insects. High surface to volume ratios combined with an open circulatory system renders insects very susceptible to desiccation. This has been overcome by the molecular configuration of certain lipids in the cuticle that form a major barrier to water loss. The utilization of triacylglycerol for metabolic and storage energy is another important result of adaptation. Triacylglycerol has several advantages over carbohydrate as a reserve energy source. Triacylglycerols have a higher caloric content per unit weight. Twice as much metabolic water is produced upon oxidation of triacylglycerol versus carbohydrate. In addition, triacylglycerols may be stored in the anhydrous form whereas carbohydrates are stored in a more bulky hydrated form (Gilbert and Chino, 1974; Downer and Matthews, 1976). This use of lipids for metabolic substrates allows the accumulation of large reservoirs of energy which may be used during periods of prolonged energy demand.

Fatty Acids

Most of the potential energy available from triacylglycerol is contained within the fatty acid component of the molecule. Fatty acids are also components of phospholipids which play essential structural and functional roles in all membrane systems of the cell (Candy, 1985). Fatty acids are precursors in the biosynthesis of waxes, pheromones and eicosanoids, and are components of defensive secretions (Stanley-Samuelson, et al., 1988).

Saturated fatty acids are synthesized by a cytoplasmic fatty acid synthetase system from acetyl-CoA, malonyl-CoA, and NADPH. Additional enzymes function to elongate and desaturate the products of the cytoplasmic system and fatty acids of dietary origin. A microsomal system elongates preformed fatty acids by successive additions of acetyl-CoA. Desaturation may also occur in the microsomal system. Several different pathways have been found within Insecta. This permits considerable variation in the fatty acid composition between and within species (Downer, 1978). Fatty acids are incorporated into tissues and then may be modified by a number of enzyme systems. These pathways of alterations carry fatty acids from one area of biological significance into another. Protoplasmic fatty acids usually vary from 2 to 34 carbons in chain length and are most commonly composed of an even number of carbons, with oleic acid (9-octadecenoic acid) being the most abundant in nature (Gilbert, 1967). Developmental stages also influence biosynthetic capacity, cofactor requirements, and the nature of the fatty acids synthesized (Downer, 1978; de Renobales, et al., 1990).

<u>Fatty Acids in Plants, Bacteria, Fungi and Animals</u>

Seven fatty acids account for almost 95% of the total fatty acids of plant tissue or most commercial seed oils (Harwood and Russell, 1984). They include both saturated fatty acids such as lauric (dodecanoic acid), myristic (tetradecanoic acid), palmitic (hexadecanoic acid), and stearic (octadecanoic acid) acids, and unsaturated fatty acids such as oleic (9-octadecenoic acid), linoleic (9,12-octadecadienoic acid), and α-linolenic (9,12,15-octadecatrienoic acids) acids. The unsaturated fatty acids all contain the *cis* Δ^9 double bond and the polyunsaturated acids have the double bond separated by a methylene group.

In contrast to animals, plants, most fungi and algae, are able to insert additional double bonds on the methyl, but not the carboxyl, side of a pre-existing double bond (Nes and Nes, 1980). Therefore, plants synthesize 9,12-octadacadienoate, while animals form 6,9-octadacadienoate from 9-octadecenoic acid (Tinoco,et al.,1979; Cripps, 1986; Harwood and Russell, 1984). The control of desaturases are not particularly well understood in any organism. In animals, the enzymes are under dietary and hormonal control. In higher plants and green algae, it is known that both light and temperature can have serious effects. Most plants and bacteria increase the proportion of unsaturated fatty acids when the environmental temperature is lowered, probably to regulate membrane fluidity.

Other photosynthetic organisms, such as green and brown algae, contain fatty acids similar to higher plants, but marine algae often contain high amounts of very-long chain polyunsaturated fatty acids such as arachidonic (5,8,11,14-eicosatetraenoic acid) and 5,8,11,14,17-eicosapentaenoic acid (Nes and Nes, 1980). Cyanobacteria are very diverse; some are 'animal-like' (containing γ-linolenic acid) while some are 'plant-like' (containing α-linolenic acid).

The major fatty acids of fungi, regardless of taxonomy, are unbranched and are usually composed mainly of palmitic, oleic, and linoleic acids. Like cyanobacteria, the higher fungi (yeast) contain α-linolenic acid while the lower fungi contain γ-linolenic acid (Nes and Nes, 1980).

In general, bacterial fatty acids are either saturated or monosaturated fatty acids of 12-20 carbons in length. Bacteria differ from most other organisms in that bacteria usually do not synthesize polyunsaturated fatty acids (Harwood and Russell, 1984).

The mammalian route of fatty acid synthesis is strongly limited by the extent of chain elongation and desaturation (Nes and Nes, 1980). Successive and nonstop condensation of the 2 carbon unit occurs only to the 18 carbon stage, and only 4 double bonds can be introduced in the 4, 5, 6, and 9 positions. The double bond does not undergo methylation, as it does in plants, and the methyl groups between the 5 carbon and the 15 carbon prevent introduction of a bond at the 9 position. The introduction of the bond at the 9 position must precede desaturation at the 6 position. Even though 2-carbon elongation only occurs as far as 18-carbons in the saturated series, once a double bond is introduced, chain elongation can occur. This permits the formation of longer fatty acids and shifts the double bonds towards the methyl end of the molecule. The fatty acids derived by direct *de novo* biosynthesis are used for membranous and other nonhormonal purposes. Therefore, animals have a dietary requirement for an acid ("essential fatty acid") that can enter the prescribed pathway for further chain elongation and desaturation. Linoleic acid is one of these because it is desaturated by a Δ^6 desaturase to γ-linolenic acid. γ-Linolenic acid may then be converted by chain elongation to 8,11,14-eicosatrienoic acid which can then be desatruated and at the 5 carbon to 5,8,11,14-arachidonic acid, a precursor for hormones. This can not be achieved with either oleic (Δ^{9}) or α-linolenic ($\Delta^{9,12,15}$) acids (Nes and Nes, 1980).

Vertebrates do not have a Δ^{12} desaturase so they are not able to place a second double bond in the 12 position of oleic acid to produce 9,12-linoleic acid. Therefore, a dietary source for this fatty acid is required for most animals (Cripps et al., 1986; de Renobale et al., 1987). Essential fatty acids have two main physiological roles: structural and hormonal. Linoleic acid is essential for maintaining proper membrane fluidity and permeability because of the structural requirements of specific membrane phospholipids for polyunsaturated fatty acids. In addition, linoleic acid is a precursor of prostaglandins, thromboxanes and leucotrienes. These recently-depicted hormone-like entities, are present in physiologically active vertebrate tissues and are involved in the regulation of a wide range of localized or cellular metabolic processes (Dadd, 1983a). Most animals and birds can satisfy this dietary requirement with linoleic or γ-linolenic acid which they elongate and further desaturate, at the carboxyl end, to the physiologically essential arachidonic acid (Dadd, 1985). Fish require α-linolenic acid which is elongated and desaturated to 5,8,11,14,17-eicosapentaenoic and 4,7,10,13,16,19-docosahexaenoic (Tinoco et al., 1979).

Fatty Acids in Insects

Generalizations about the fatty acid compositions of insects as a group are difficult to make. Previous evidence suggested the existence of a basic similarity of insects with mammals in the biosynthesis of fatty acids as far as oleate for nonhormonal purposes and also the requirement for exogenous unsaturated fatty acids for hormonal and other specialized purposes (Nes and Nes, 1980). Fatty acid compositions reflect endogenous fatty acid metabolism in insects and the list of species that appear to exhibit unconventional patterns of lipid metabolism is growing rapidly (Stanley-Samuelson, 1984). Growth and development have proven to have strong influences on the fatty acid profiles of many insects (Barnett and Berger, 1970; Cripps et al., 1988). Seven fatty acids represent the major proportion of the fatty acid complement of most insects. The prevalent saturated fatty acids are myristic acid, palmitic acid, and stearic acid. The common monounsaturated fatty acids are palmitoleic acid and oleic acid. The polyunsaturated acids present are linoleic and α-linolenic acid (Dadd, 1983a).

As in vertebrates, the polyunsaturated fatty acids identified in insects are proving to be important molecules functioning not only in membrane structure but as precursors of prostaglandins and probably other oxygenated eicosanoid products as well (Nes and Nes, 1980). By the introduction of a second double bond into oleic acid, at least 15 insect species are able to biosynthesize linoleic acid (Blomquist, et al.,1982; Cripps et al.,1986). This was previously believed not to occur in any animal cell. In this single step, a monounsaturated fatty acid is converted into a polyunsaturate. Metabolic interconversions of polyunsaturated fatty acids allow compositions of particular membranes to be tailored to specific local requirements which may differ according to tissue, age, reproductive status, and season (Stanley-Samuelson, et al., 1988).

There has been much controversy on the subject of biosynthesis of polyunsaturates in insects. Nutritional studies of the 1950's and 1960's demonstrated that linoleate was essential for more than half of the species studied (Dadd, 1985). The apparent lack of a requirement for dietary linoleic acid for some species was often attributed to contaminated artificial diets that had enough linoleic acid to satisfy growth requirements. Other explanations were offered even when early radiotracer experiments showed incorporation of radiolabeled acetate into a fraction which coeluted with a linoleate standard by gas chromatography (Louloudes, et al., 1961; Strong, 1963a; Mauldin, 1972). One suggestion was that the analytical techniques utilized were not adequate to separate linoleate from other 18 carbon fatty acids. This would result in small amounts of radioactivity in linoleate fractions. Another suggestion was that the labelled dienoic acid could have been the $\Delta^{6,9}$ isomer (the 'animal' diene) rather than the $\Delta^{9,12}$ isomer (the 'plant' diene) since no attempt was made to locate the positions of the double bonds. Finally, it was suggested that symbiotic microorganisms did it (de Renobales, et al., 1987).

Several analytical techniques have now been used to show that certain insects apparently do synthesize linoleic acid. These techniques included gas chromatography, high performance liquid chromatography, silver nitrate thin-layer chromatography, and the confirmation of positions of the double bonds by gas chromatography-mass spectrometry and ozonolysis (Blomquist, et al., 1982; Cripps, et al., 1986; de Renobales, et al., 1986; Jurenka, et al., 1987). The role of symbiotic microorganisms have been examined by several approaches including using tissue other than gut tissue, defaunating the insect by injecting antibiotics, and removal of the gut tract (Blomquist, et al., 1982; de Renobales et al., 1986).

In all cases, the treated insects synthesized linoleic acid at the same levels as the untreated controls. Biosynthesis of linoleic acid by bacteria from the mycetocytes is unlikely because the *de novo* synthesis of a diunsaturated fatty acid with non-adjacent double bonds by bacteria has never been verified (Harwood and Russell, 1984; de Renobales, et al., 1987)

Linolenic Acid

Occurrence and Function

α-Linolenic acid along with linoleic acid are the major acyl moieties found in thylakoid membrane lipids in all plant chloroplasts and therefore plants are the principal sources of these polyunsaturated fatty acids (Stumpf, 1981). α-Linolenic acid is also found in fish and many insects.

γ-Linolenic acid is found primarily in animals since they can only desaturate on the carboxyl side of the Δ^9 double bond. Ingested linoleic acid is desaturated at the Δ^6 to γ-linolenic acid which may be incorporated into membranes or elongated and again desaturated to arachidonic acid, a precursor of prostaglandins and their derivatives. It is thought that some insects may have both the Δ^6 and Δ^{15} desaturases and so are able to biosynthesize both α and γ-linolenic acid (Stanley-Samuelson, et al., 1987).

For many Lepidoptera, linoleic and/or linolenic acids are essential for proper adult development. Over 40 years ago, Fraenkel and Blewett (1945) showed that linolenic acid was far superior to linoleate in preventing naked and deformed wings, as well as deformed moths. Linolenate was also shown to increase growth rates. In the wax moth, Galleria mellonella, α-linolenic acid appeared to be 10-fold more potent in alleviating faulty adult development than linoleic acid (Dadd, 1983b). The fatty acids required for adult development differ substantially among insect species. Nutritional studies with 18 species of Lepidoptera revealed that 3 of them had no apparent fatty acid requirement for pupal/adult ecdysis, 9 utilized either linoleic or linolenic acids, 5 required linolenic specifically, and one species required both fatty acids (Dadd, 1983a). Apart from that both fatty acids are required for normal growth and development. Most papers, especially before 1980, did not mention or know the which isomer of linolenic acid was used in these studies.

A review by Fast (1964) indicated that most researchers had found linolenic acid present in Coleoptera but failed to quantify it. There were no indication as to which isomer was present and no GC-MS comfirmation for any linolenic acid in this review. Lepidoptera were generally reported as having high percentages of linolenic and/or oleic acids (18.5 to 60.1%). Linolenic acid was reported absent in all Homoptera. There were reports of three Orthoptera containing linolenic acid (6.9, 37.4% and not quantified) while the others reported it absent. Linolenic acid in Diptera were reported as absent or present but not quantified. Linolenic acid was listed as absent in honey bees and an ant but present and not quantified in other Hymenoptera. The American cockroach (Dictyoptera) was reported as containing 4.3 % of linolenic acid for the males and 4.6 % for the females. Linolenic acid was reported as present but not quantifed in a Neuroptera.

In a later review by Fast (1970), 3 out of 270 sources indicated that the linolenic acid was the α isomer (9,12,15-18:3) (Keith, 1966; Nelson and Sukkestad, 1968; Stephen, Jr. and Gilbert, 1970). In this review, Coleoptera were reported as having varing amounts of linolenic acid; from absent to 23.1 % for total lipids, 36.1 % in the neutral lipids, and from 5.9 to 37.5 % in the phospholipids. Most Lepidoptera, as in his first review, were reported containing high linolenic and/or oleic acids in the total lipids but mostly high linolenic acid in the phospholipids. Linolenic acid was either not looked for, reported as absent or very low percents (>1.7 %) in the total lipids of the Heteroptera but reported percentages of 1.5 to 36 % in the phospholipid classes. The Orthoptera were reported as having varying amounts of linolenic acid from 1 to 43 % in the total lipid and absent to 30.8 % in the phospholipid classes. Linolenic acid was not looked for in the total lipid in most Diptera while a few were reported as containing >5 % except for a Tipulidae with 14.8 %. The phospholipids of the Diptera were reported as containing 2.2 to 24.9 % linolenic acid. Linolenic acid was listed as being absent in the total lipids of honey bees and ants and 1.5 to 36.3 % in other Hymenoptera. The phospholipids of Hymenoptera contained 13.2 to 37 % linolenic acid. The American cockroach (Dictyoptera) contained 0.8 to 3.2 % linolenic acid in the phospholipids. This differs from a review by Thompson (1973) which states that Dictyoptera lack polyunsaturates including linolenic acid.

Structural studies were done by very few researchers before the 1980's. Keith (1966) chemically cleaved the double bonds of unsaturated fatty acids from Drosophila melanogaster with $KHMnO_4$. The position of double bonds in fatty acid methyl esters were determined by ozonolysis , reductive cleavage of the ozonide, and GC analysis by Nelson and Sukkestad (1968). Nelson and Sukkestad were working with the cabbage looper. Later structural studies were conducted by ozonolysis and GC-MS on fatty acids from a termite, cockroach, and cricket (Blomquist, et al., 1982; Jurenka, et al., 1987) and ozonolysis and radio-GC on fatty acids from the pea aphid (de Renobales, et al., 1986). Stanley-Samuelson, et al., (1990) conducted analyses by capillary GC-Electron Impact MS to confirm the FAMEs in Microdon albicomatus and Myrmica incompleta. These conbined analyses showed the carbon chain length and double bond placement in the polyunsaturated fatty acids including linolenic acid.

With the development of instruments that can measure minute amounts of material and new and improved methods, literature is being outdated daily. The present literature does not adequately record the occurrence and distribution of the isomers of linolenic acid. Many references do not know or refer to which linolenic acid isomer is present, and others assume there is only the α isomer in insects.

CHAPTER III

MATERIALS AND METHODS

Insects

Insects were collected or obtained from a number of sources (Table I). Season refers to when insects were collected in the field, the other insects were reared in controlled environments.

Extraction of Total Lipid

Chloroform, hexane, and methanol were glass redistilled from laboratory grade chemicals obtained from Sigma Chemical Co. (St. Louis, Mo.). Live insects were weighed and either immediately homogenized or frozen for later use. Lipids were extracted and purified using the method of Bligh and Dyer (1959). In an ice bath, whole insects were homogenized in 0.8 ml of water, 2.0 ml of methanol and 1.0 ml of chloroform. The homogenate was transferred to a centrifuge tube and 1.0 ml of water and 1.0 ml of chloroform added, vortexed and then centrifuged for 10 min. The chloroform subphase was removed with a pasteur pipette and retained in a 7 ml glass scintillation vial. Another 1.0 ml of chloroform was added to the homogenate, vortexed, and again centrifuged for 10 min. The subphase was again removed, combined with the first, and the

TABLE I

INSECTS ANALYZED

INSECT	SOURCE	SEASON	LIFE STAGE
Chrysanthemum Aphids *Macrosiphoniella sanborni* (Gillette)	Chyrsanthemum *Chrysanthemum* sp.	late April	mixed
Giant Bark Aphids *Longistigma caryae* (Harris)	Shumard Oak *Quercus shumardii* Buckl.	April	mixed
Blue Alfalfa Aphids *Acyrthosiphon kondoi* Shinji	Alfalfa *Medicago sativa* L. cv 'OK08'	Colony*	mixed
Citrus Mealybugs *Planococcus citri* (Risso)	Alfalfa *Medicago sativa* L. cv 'OK08'	Greenhouse	mixed
Spotted Alfalfa Aphids *Therioaphis maculata* (Buckston)	Alfalfa *Medicago sativa* L. cv 'OK08'	Colony*	mixed
Alfalfa Leafworms *Heliothis zea* Boddie	Alfalfa *Medicago sativa* L. cv 'OK08'	early June	larval
Alfalfa Weevils *Hypera postica* (Gyllhal)	Alfalfa *Medicago sativa* L. cv 'OK08'	early May	adults
Spotted Cucumber Beetles *Diabrotica undecimpunctata howardi* Barber	Alfalfa *Medicago sativa* L. cv 'OK08'	early June	adults
Spotted Cucumber Beetles *Diabrotica undecimpunctata howardi* Barber	Okra *Hibiscus esculentus* L. Green beans *Phaseolus vulgaris* L.	early July	adults

TABLE I (Continued)

INSECT	SOURCE	SEASON	LIFE STAGE
Greenbugs *Schizaphis graminum* Rondoni	Wheat *Triticum aestivum* L. cv 'Triumph'	Colony*	mixed
Bird Cherry-Oat Aphids *Rhopalosiphum padi* L.	Wheat *Triticum aestivum* L. cv 'Triumph'	Colony*	mixed
Russian Wheat Aphids *Diuraphis noxia* Nordvilko	Wheat *Triticum aestivum* L. cv 'Triumph'	Colony*	mixed
English Grain Aphids *Sitobion avenae* (Fab.)	Wheat *Triticum aestivum* L. cv 'Triumph'	Colony*	mixed
Lesser Grain Borers *Rhyzopertha dominica* (Fab.)	Wheat *Triticum aestivum* L. seed	Colony*	adults
Turnip Aphids *Lipaphis erycimi* Kaltenbach	Turnip *Brassica rapa* L.	Colony*	adults
Yellow Sugarcane Aphids *Sipha flava* (Forbes)	Sorghum *Sorghum bicolor* (L.) Moench cv 'SGY-850'	Colony*	mixed
C7 Lady Beetles *Coccinella septempunctata* L.	Sorghum *Sorghum bicolor*	late June	adults
Spotted Lady Beetles *Coleomegilla maculata* Tamberlake	Sorghum *Sorghum bicolor*	late June	adults
Oleander Aphids *Aphis nerii* Boyer de Fonscolombe	Climbing Milkweed *Cynanchun laeve* (Michx.) Pers.	mid-August	mixed

TABLE I (Continued)

INSECT	SOURCE	SEASON	LIFE STAGE
Black Pecan Aphids *Melanocallis caryaefoliae* (Davis)	Pecan *Carya illinoensis* (Wangenh.) K. Koch	early September	mixed
Fall Webworms *Hyphantria cunea* Drury	Pecan *Carya illinoensis* (Wangenh.) K. Koch	July	larval
Fall Webworms *Hyphantria cunea* Drury	River Birch *Betula nigra* L.	June	larval
Birch Aphids *Calaphis betulla* Walsh	River Birch *Betula nigra* L.	July & August	mixed
Pea Aphids *Acyrthosiphon pisum* (Harris)	Broadbeans *Vicia Faba* L. cv 'Windsor'	Colony*	mixed
Small Milkweed Bugs *Lygaeus kalmii* Stal	Broadleaf Milkweed *Asclepias latifolia*	August	adults
Milkweed Tiger Moths *Euchaetes egle* (Drury)	Broadleaf Milkweed *Asclepias latifolia*	June	larval
Black Scales *Saissetta oleae* (Olivier)	Ornamental Figs *Fica* sp.	Late July	mixed
Bagworms *Thyridopteryx ephemeraeformis* (Haworth)	Eastern Red Cedar *Juniperus virginiana* L.	early July	larval
Yellow-stripped Armyworms *Spodoptera ornithogalli* Guenée	Dock *Rumex* sp.	early June	larval

TABLE I (Continued)

INSECT	SOURCE	SEASON	LIFE STAGE
White-lined Sphinx *Hyles lineata* (Fab.)	Dock *Rumex* sp.	early June	larval
Squash Bugs *Anasa tristis* (DeGeer)	Yellow Squash *Curcurbita pepo* L. cv 'Melopepo'	Colony*	1st instar
Southern Masked Chafers *Cyclocephala immaculata* (Olivier)	at light	early June	adults
Green Lacewings *Chrysopa* sp.	at light	early June	adults
Horse Fly *Tabanus abactor* Philip	Gurnsey Cow *Bos taurus* L.	early June	adults (♀)
Horse Fly *T. atratus* Fab.	Gurnsey Cow *Bos taurus* L.	early June	adults (♀)
Common Meadow Grasshopper *Conocephalus f. fasciatus* (DeGeer)	Sweeping native grass	early June	adults
Field Cricket *Gryllus* sp.	Cracks in ground	early September	adults (♀)
Honey Bees *Apis mellifera* L.	Local Hive	mid-August	adults (workers), pupae and larval
German Cockroach *Blattella germanica* (L.)	Dog food	Colony*	adults

TABLE I (Continued)

INSECT	SOURCE	SEASON	LIFE STAGE
American Cockroach *Periplaneta americana* (L.)	Dog food	Colony*	nymphals and adults
Large Milkweed Bug *Oncopeltus fasciatus* (Dallas)	Sunflower seeds+	Colony*	adults
Dark Mealworms *Tenebrio abscurus* Say	Bran meal+	Colony*	larval
House Flies *Musca domestica* L.	Sugar, powdered milk and wood shavings+	Colony*	larval and adults (♀ & ♂)
Blow Flies *Sarcophaga bullata*	Liver+	Colony*	larval and adults
Greater Wax Moth *Galleria mellonella* (L.)	Honey comb+	Colony*	larval
Tobacco Hornworm *Manduca sexta* (L.)	Artificial diet+	Colony*	larval

* Established colonies reared at controlled temperature and light

+ Carolina Biological Supply Co., Burlington, NC chloroform was evaporated under nitrogen. If the sample was not to be divided, 100 μl of heptadecenoic acid methyl ester (1 μg/μl) was added as an internal standard and dried under nitrogen. Samples were stored in 1.0 ml of chloroform containing .05% butylated hydroxytoluene (BHT) (w:v) at -20° C. Samples were split into 2 fractions for the analysis of total lipids (TL) and separation of neutral (NL) and polar (PL) lipids. (Since polar lipids contain mostly phospholipids, PL will refer to phospholipids in the results and discussion sections.) To do this, samples were dried down under nitrogen and then redissolved in 1.5 ml of chloroform. For TL analysis, 0.5 ml of sample was removed and combined with 100 μl of methylheptadecanoate (17:0, internal standard) prior to storage at -20° C. The remaining sample (1.0 ml) was separated into NL and PL fractions.

Separation of Neutral and Polar Lipids

NL and PL were separated by column chromatography (Lynch and Thompson, Jr., 1984). A 50 ml biuret with a teflon stop-cock was used for the column. A plug of glass wool was used to retain the absorbent. A slurry of 2.0 g of Bio-Sil A (Bio-Rad, Richmond, CA) and 30 ml of chloroform:methanol (1:1, v:v) was poured into the column and then washed with an additional 30 ml of chloroform:methanol (1:1, v:v). The top of the silica gel bed was marked on the column for a reference point. If the solvent was allowed to go below this point channels developed through the gel and the column was repacked. Prior to sample addition, the column was rinsed with 30 ml of chloroform. The sample was added to the column and NL were eluted with 30 ml of chloroform. PL were eluted with 30 ml of chloroform:methanol (1:1, v:v). The column was repacked with new silica gel for each different species of insect. Solvents were evaporated from samples under nitrogen and methylheptadecanoate (100 μl) was added to each fraction prior to storage in 1.0 ml chloroform w/BHT at -20° C.

Fatty Acid Analysis

Lipid samples, either TL, NL, or PL, were transferred to 7.0 ml glass scintillation vials and dried under nitrogen. Lipids were hydrolyzed with 1.0 ml of 5% KOH in methanol (w:v) was added. The vials were capped with teflon caps, vortexed and heated at 60° C for 90 min. After the vials were cooled, 1.0 ml of BF_3 (14% in methanol, Sigma Chemical Co., St. Louis, MO) was added and the contents vortexed and heated again at 60°C for 30 min. This methylated the fatty acids. The reaction was stopped by the addition of 2.0 ml of water. The samples were then extracted with 2.0 ml of chloroform three times. The water was removed from the chloroform by running it through a pasteur pipette containing magnesium sulfate. The chloroform was evaporated under nitrogen and 200 μl of hexane was added to the sample. The sample in hexane was then loaded onto a pasteur pipette minicolumn of Bio-Sil A. The hydrocarbons were eluted with 3.0 ml of hexane. Fatty acid methyl esters (FAMEs) were eluted with 6.0 ml of 5% ether in hexane (v:v), dried under nitrogen and redissolved with 100 μl of chloroform prior to GC analysis.

The FAMEs were analyzed with a temperature-programmed HP 5890 gas chromatograph equipped with a DB-225 capillary column (J. & W. Scientific, Deerfield, IL). The temperature program was as follows: 2 min at 120°C, then an increase of 10°C per min for 8 min until 200°C was reached, followed by an increase of 5°C per min until 225°C was reached and 225°C was held for 4 min. The chromatograms were filed data was analyzed by computer with Maxima (Millipore).

An injection of standard fatty acid methyl esters, obtained from Sigma Chem. Co. (St. Louis, Mo.), was made on the GC each day that samples were run. These standards included methyl esters of caprylate (8:0), caprate (10:0), laureate (12:0), myristate (14:0), myristoleate (14:1), palmitate (16:0), palmitoleate (16:1), stearate (18:0), oleate (18:1), linoleate (18:2), α-linolenate (α18:3), γ-linolenate (γ18:3), arachidate (20:0), gondoate (20:3), arachidonate (20:4), eicosapentaenoate (20:5), and behemate (22:0) (Fig. 2). The internal standard, methyl ester of heptadecanoate (17:0) (Fig. 3) was added at a concentration of 1 μg/μl. This was used to determine the amount of fatty acid methyl esters relative to live weights of the samples. The purified β-linolenate was mixed with α and γ-linolenate and run as a standard (Fig. 4). Fig. 5 shows the elution of the δ and β isomers obtained from aphids. Samples were analyzed by Dr. Ralph Howard, USDA Grain Marketing Laboratory, Manhattan, KS, by gas chromatography-mass spectrometry (GC-MS) to confirm structures of 18:3 isomers.

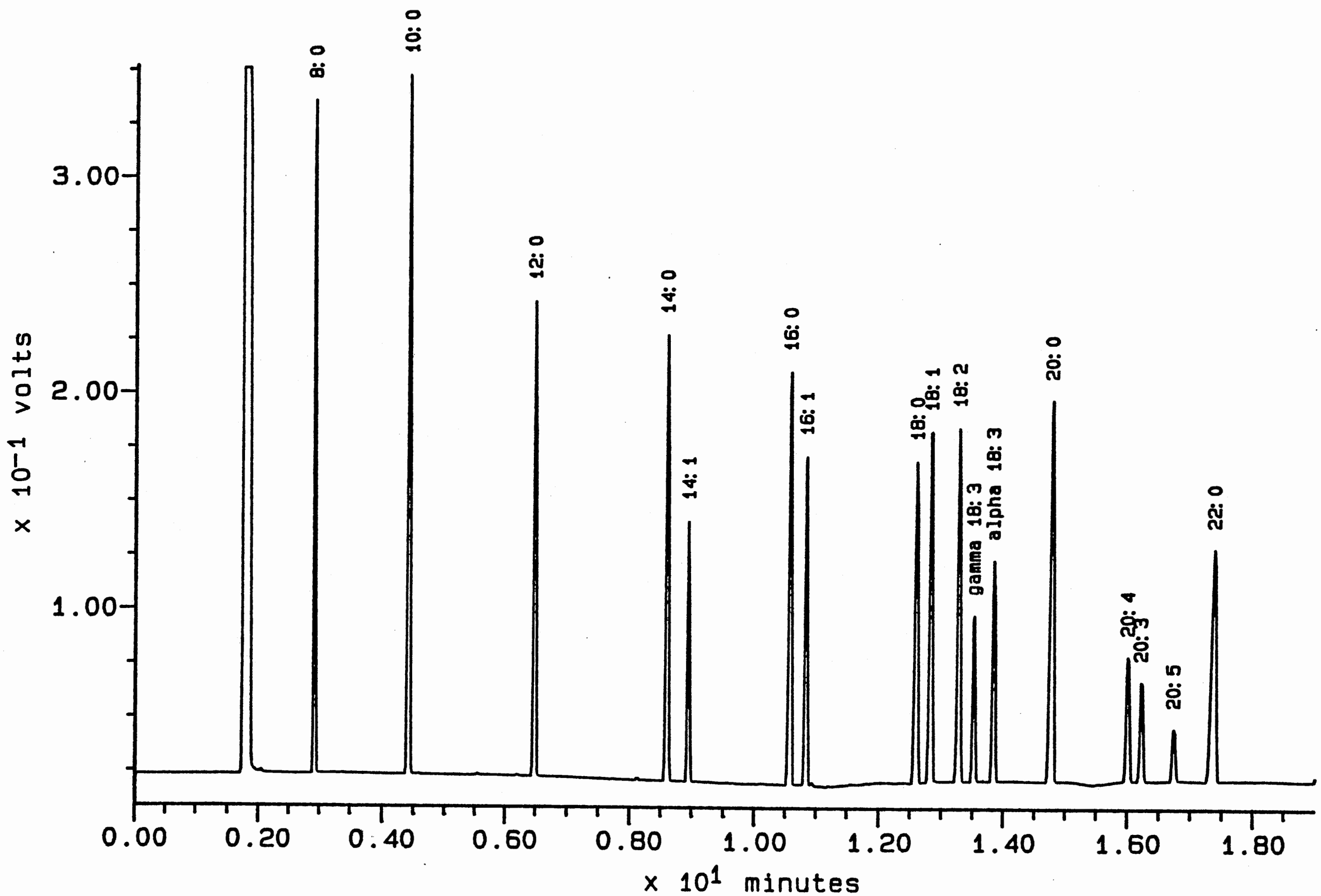


Figure 2. GC Trace of the Standard FAMEs

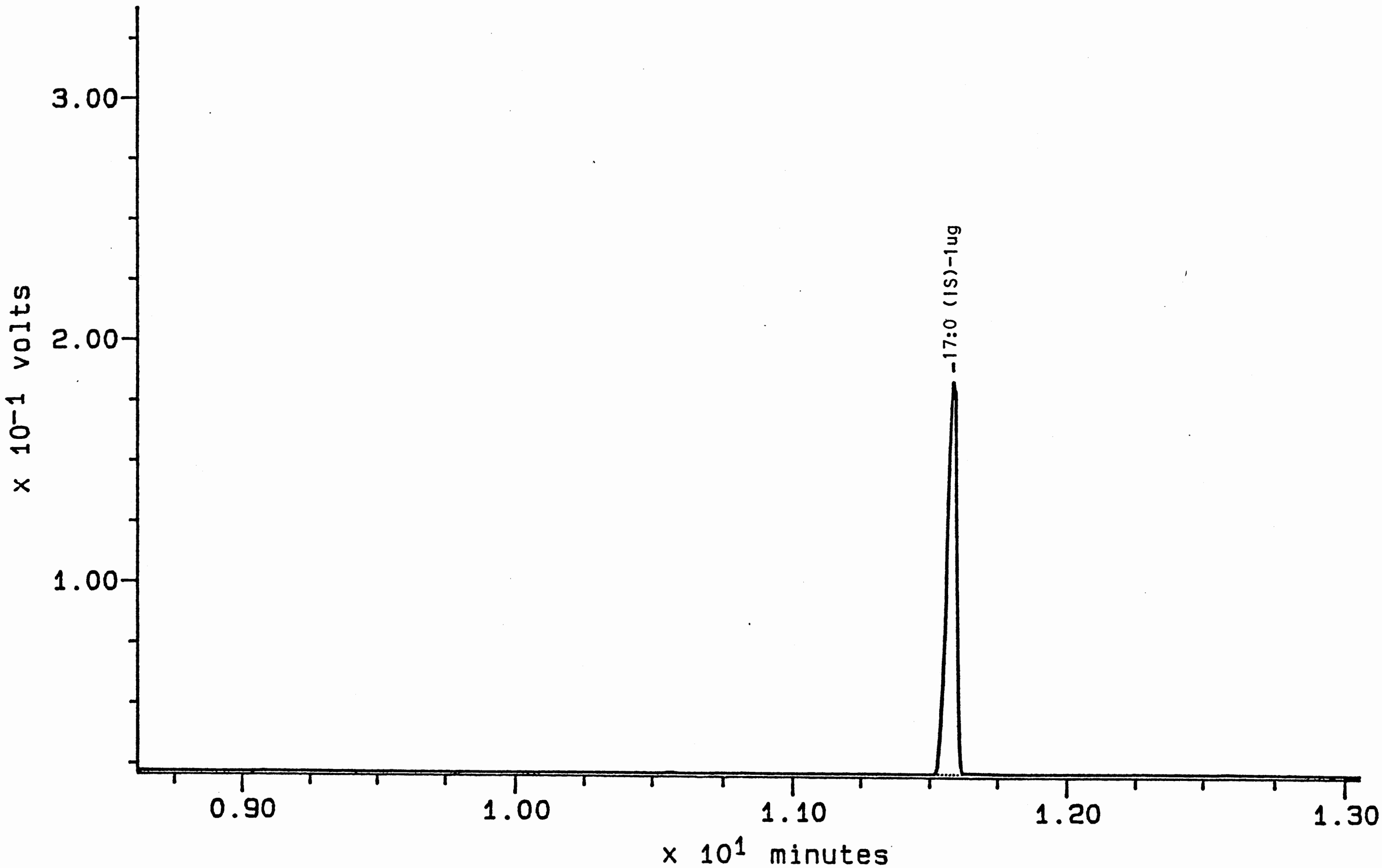


Figure 3. GC Trace of the Internal Standard, 17:0

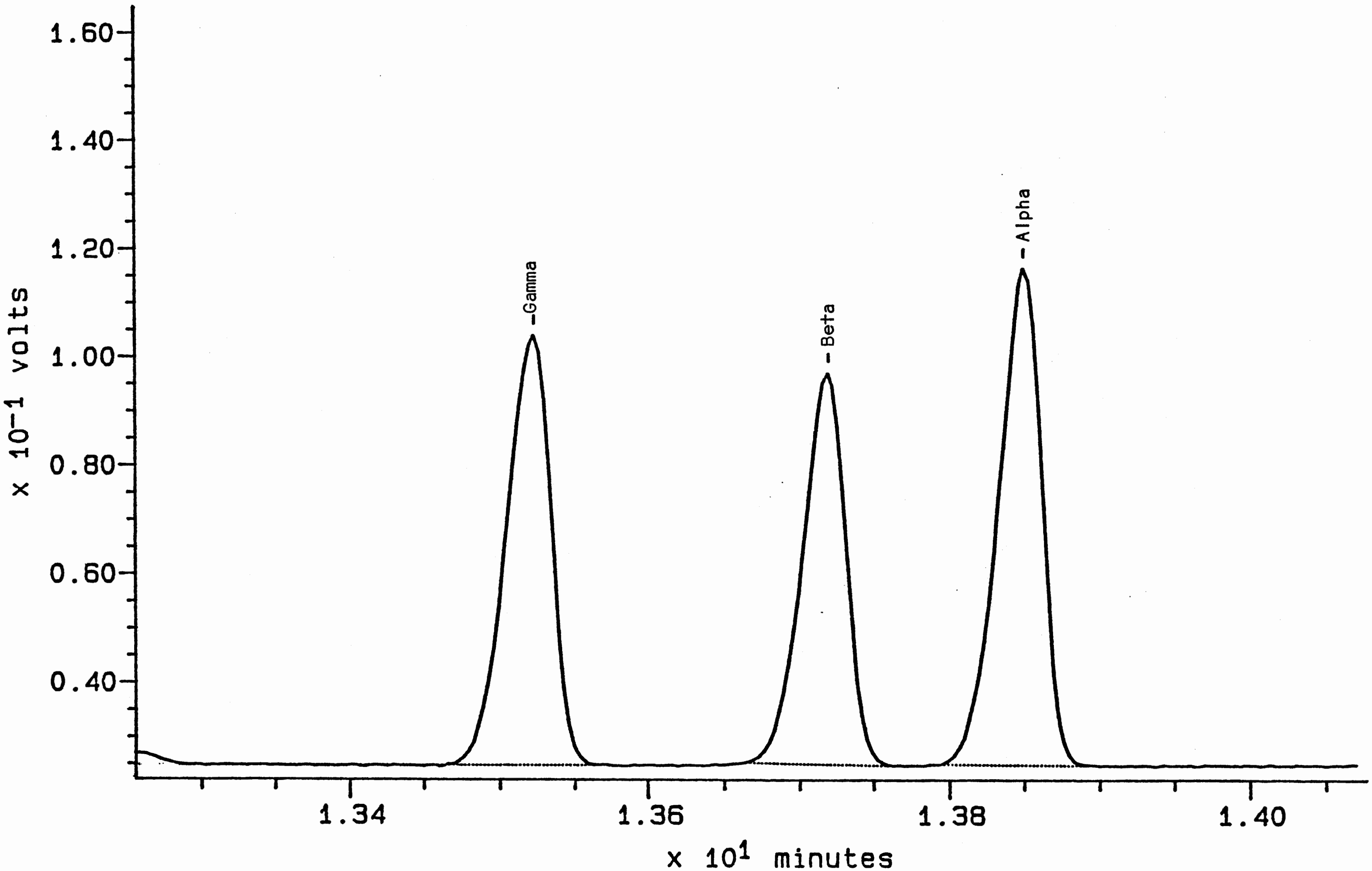


Figure 4. GC Trace of the Standard FAME of the Linolenic Acid Isomers

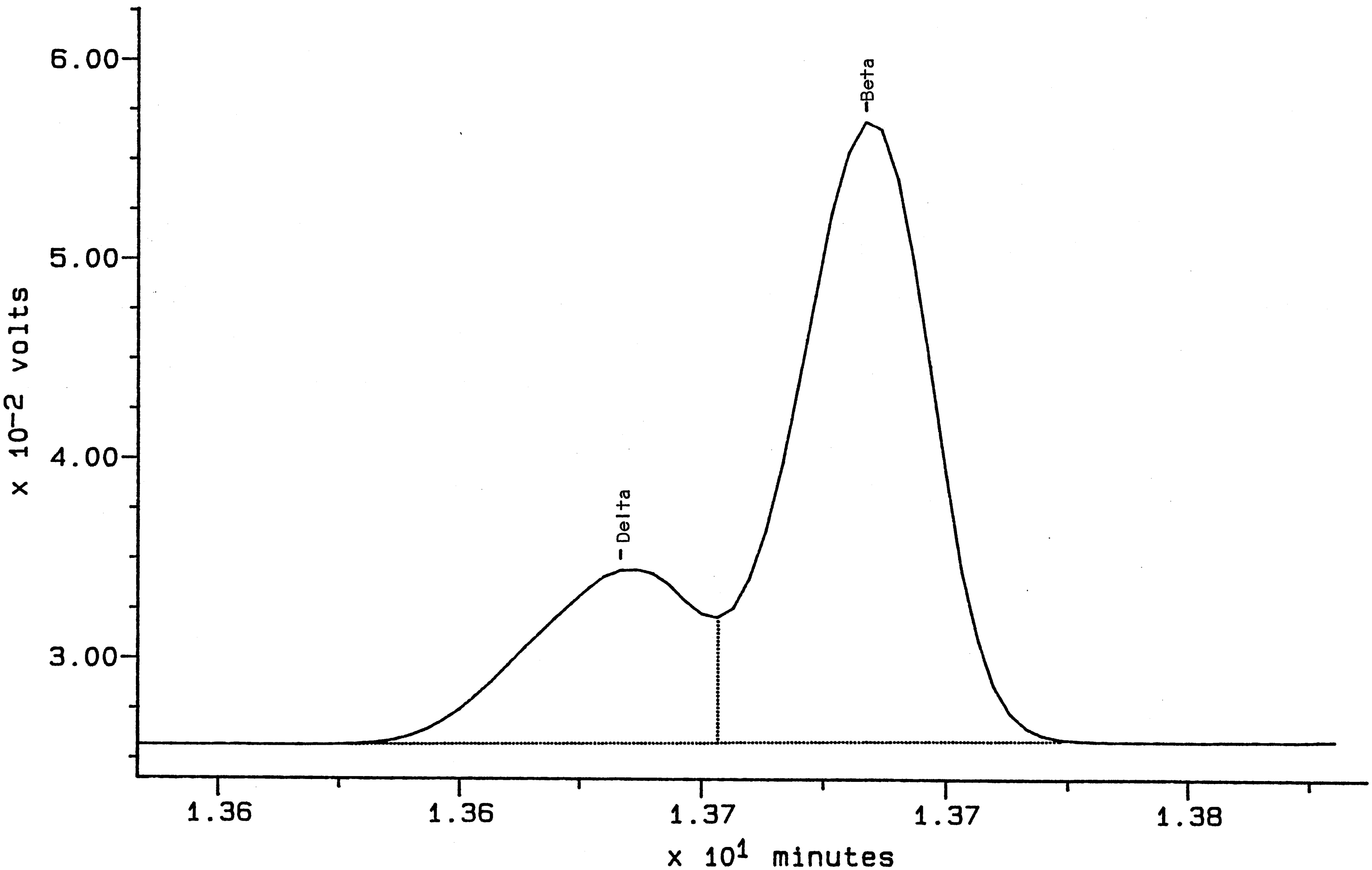


Figure 5. GC Trace of an 18:3 FAME Area of Aphids Showing the Beta and Delta Isomers

CHAPTER IV

RESULTS

Linolenic Acid Isomers of Aphids

Analysis of 18:3 isomers by GC-MS show that the δ isomer co-eluted with 19:0-methyl ester and the β isomer with 19:1-methyl ester on the columns used in this study. Therefore it was necessary to use mass spectrometry to confirm structures of these isomers. The structures of α and γ isomers were also confirmed by GC-MS.

The linolenic acid isomers occurred in the total lipid (TL) and the phospholipid (PL) but usually not in the neutral lipid (NL) (Fig. 6 & 7). Therefore, chromatograms of fatty acids from the PL fraction for the aphids will be included in this thesis (Fig. 9-21). There were not enough Chrysanthemum Aphids to split the sample, so that chromatogram is of TL (Fig. 8).

All aphids analyzed contained both β and δ linolenic acid isomers but no γ isomer (Fig. 8-21). Only the black pecan aphid contained the α isomer (Fig. 16). In terms of total linolenic acid, most aphids had similar percentages of β and δ isomers which ranged of 77.9 to 90.8% and 10.2 to 22.1% respectfully (Table II). In comparison to the rest of the fatty acids, percentages of linolenic acid isomers as well as μg/mg aphid were very small (Tables III, V, VI, & VIII). Almost all

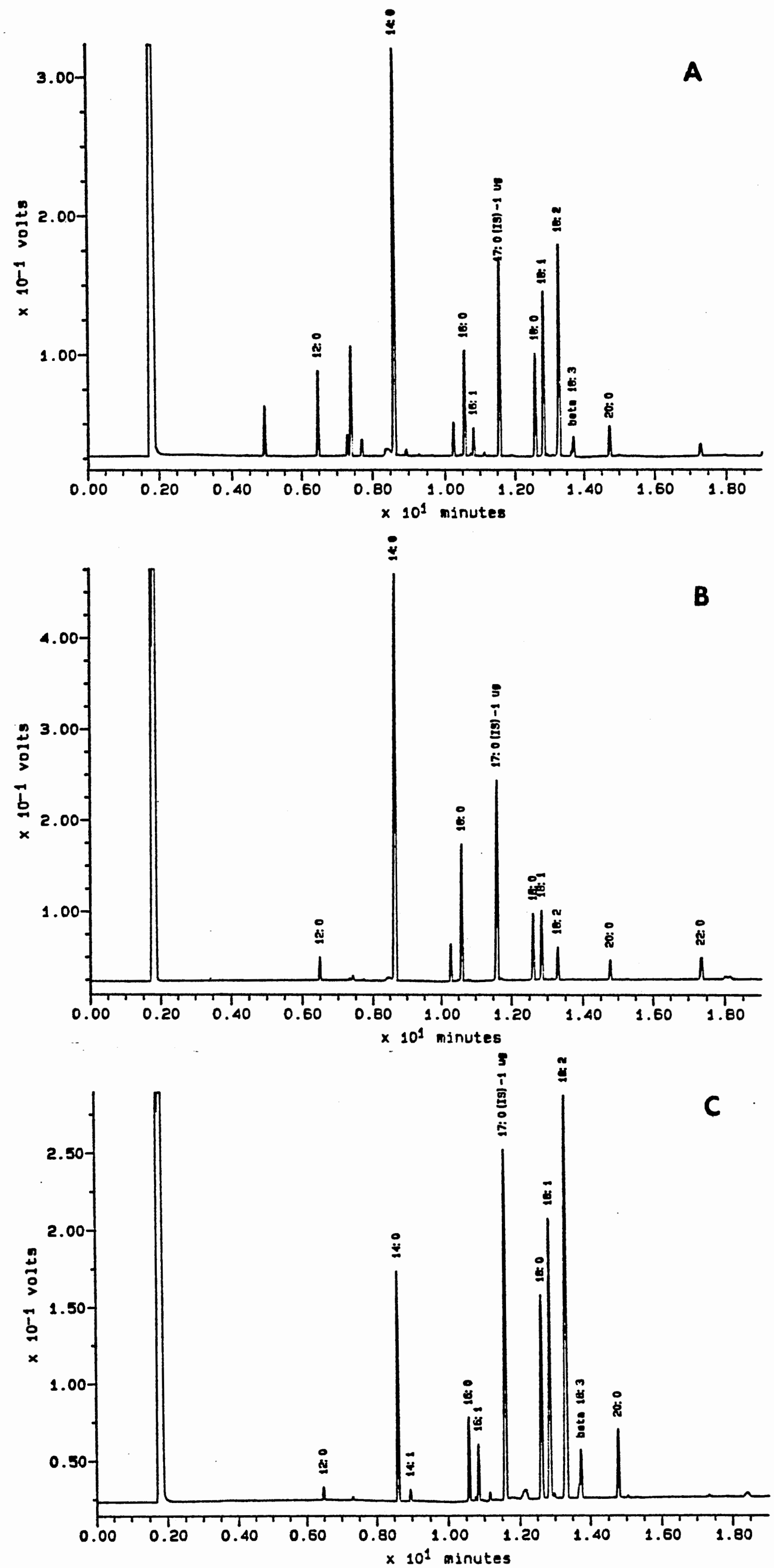


Figure 6. GC Traces of the Total (A), Neutral (B), and Phospholipid (C) FAME of the Giant Bark Aphid

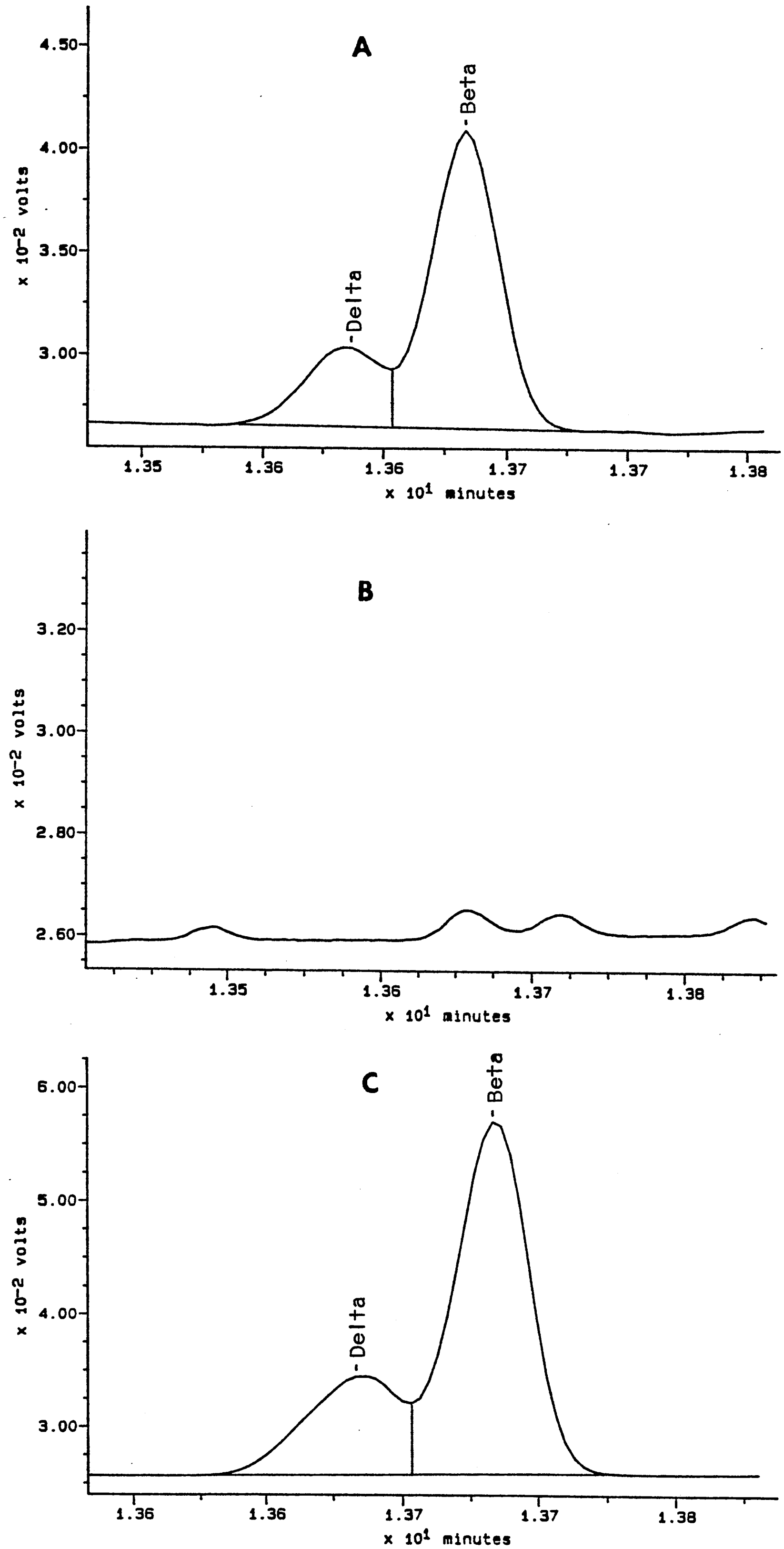
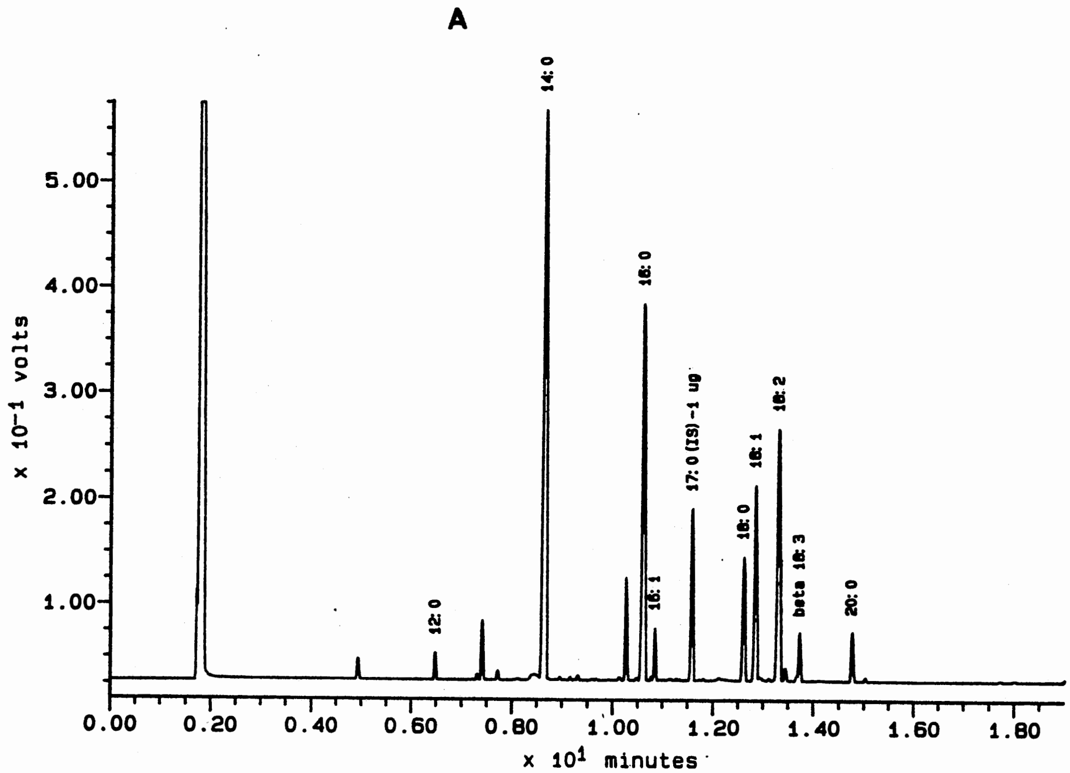


Figure 7. GC Traces of the Total (A), Neutral (B), and Phospholipid (C) FAME of the 18:3 Areas of Fig. 6



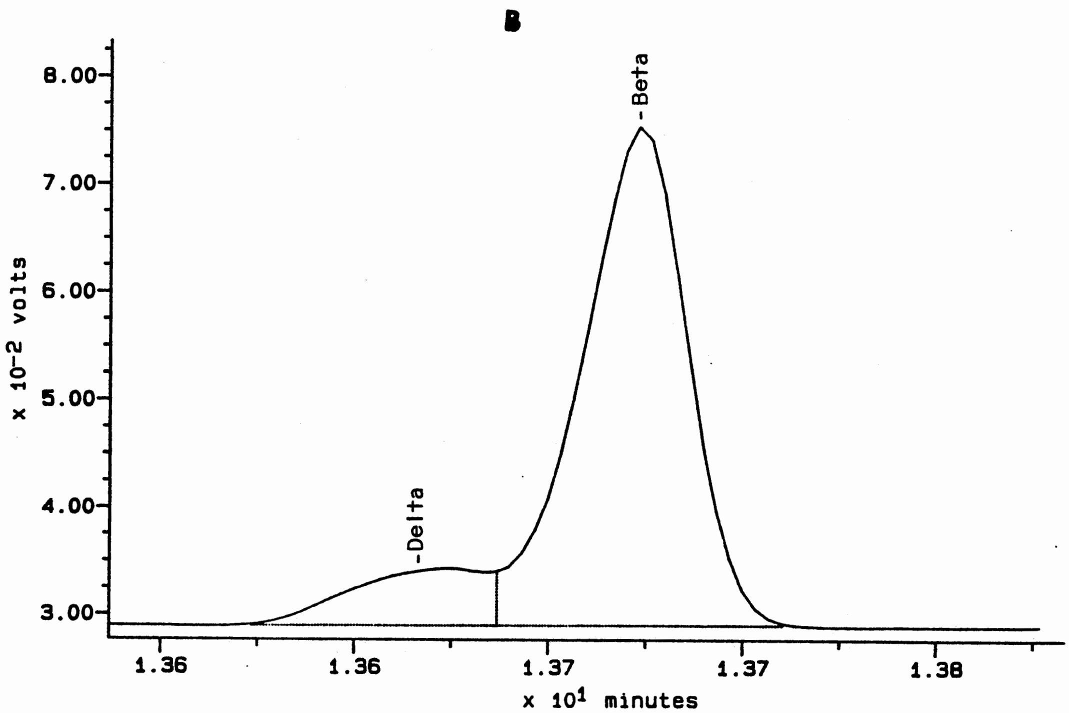


Figure 8. GC Trace of the Total Lipid FAME of the Chrysanthemum Aphid (Macrosiphoniella sanborni) (A) and the 18:3 Area of that Trace (B)

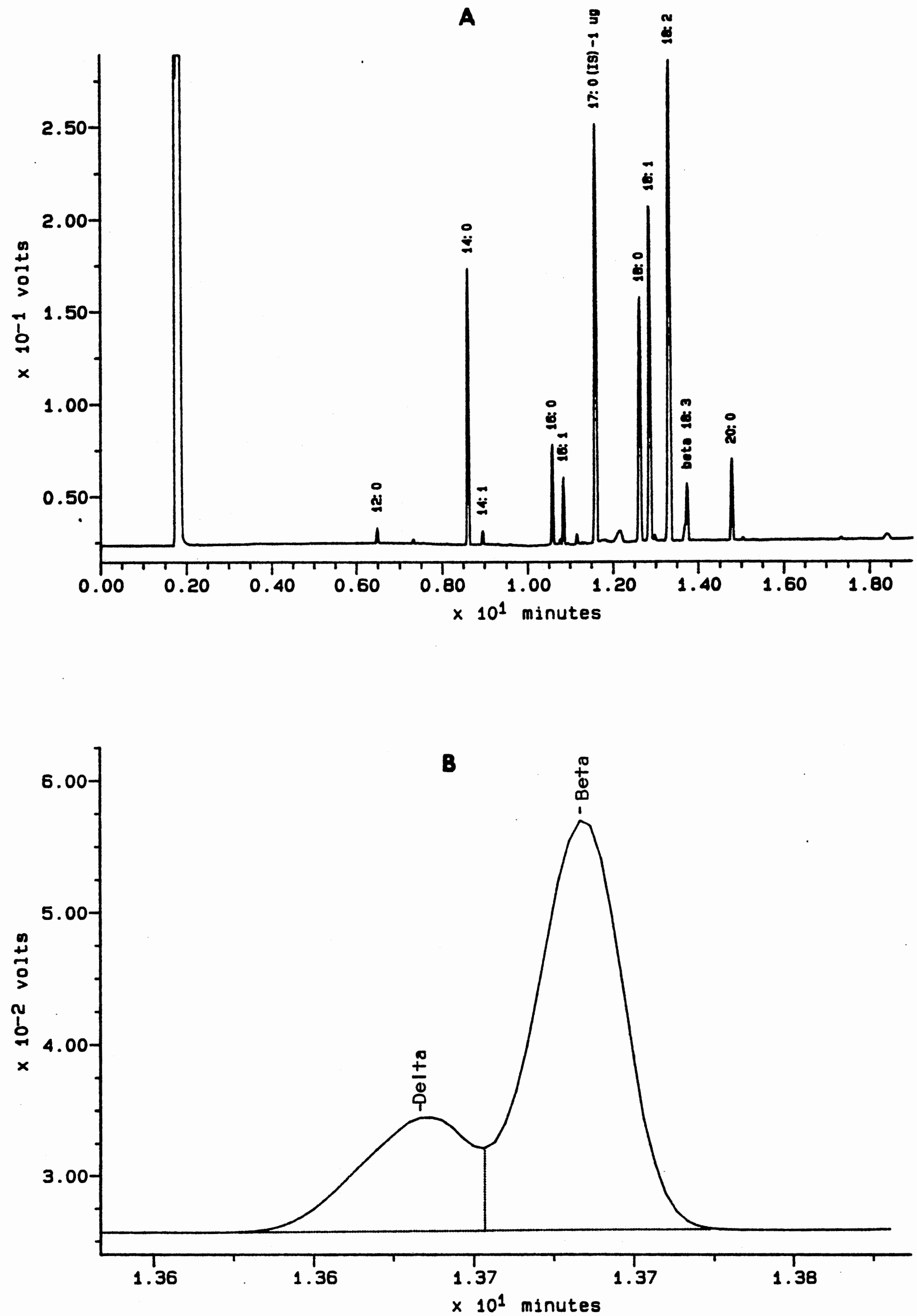
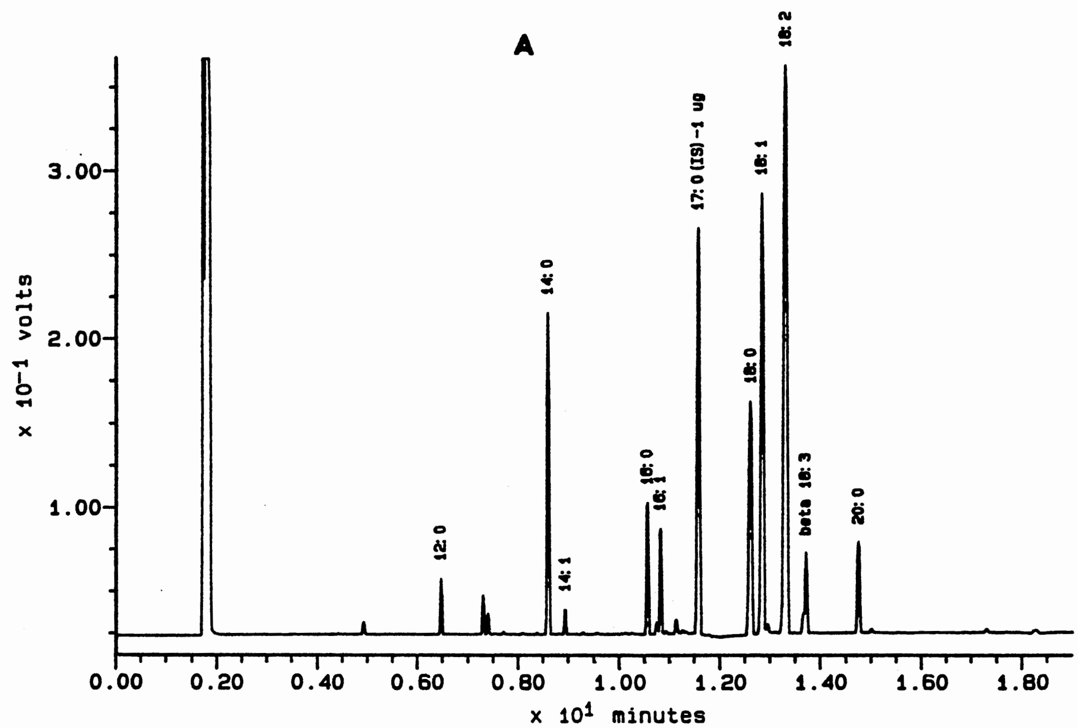


Figure 9. GC Trace of the Phospholipid FAME of the Giant Bark Aphid (Longistigma caryae) (A) and the 18:3 Area of that Trace (B)



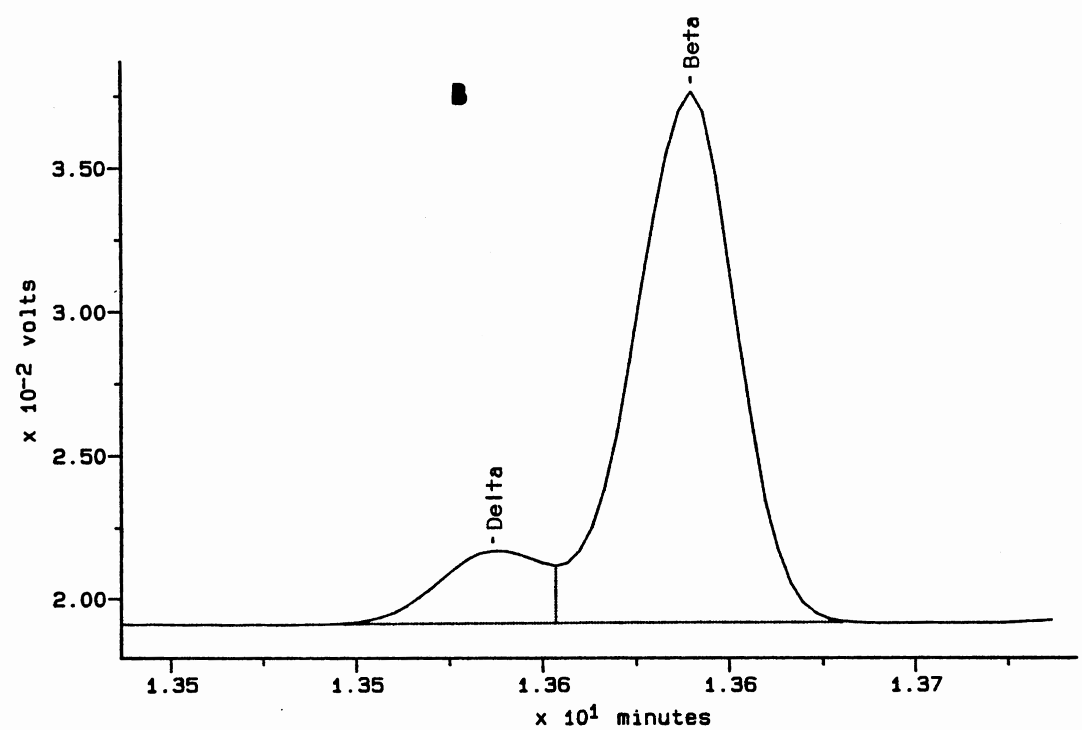


Figure 10. GC Trace of the Phospholipid FAME of the Blue Alfalfa Aphid (Acyrthosiphon kondoi) (A) and the 18:3 Area of that Trace (B)

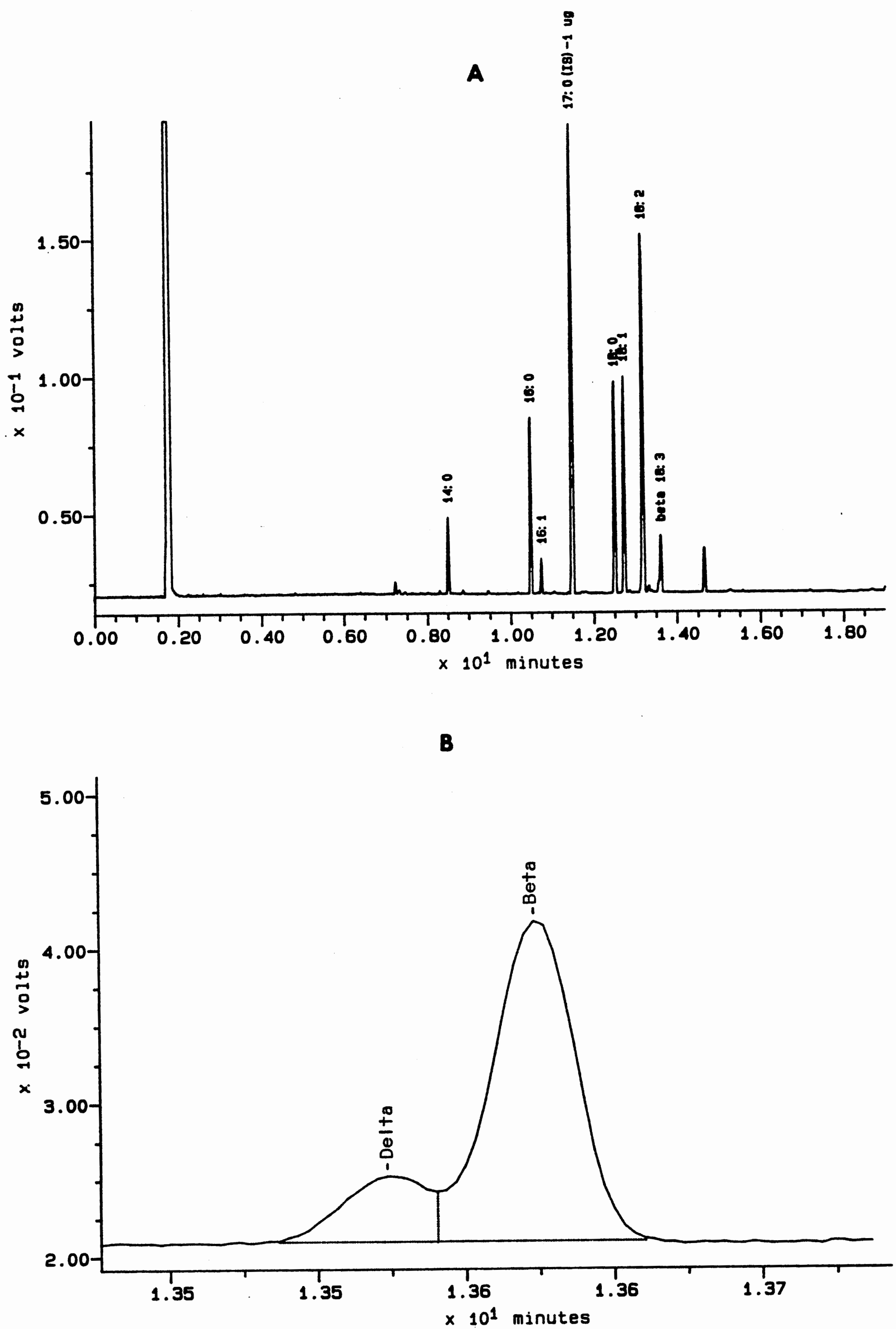


Figure 11. GC Trace of the Phospholipid FAME of the Birch Aphid (Calaphis betulla) (A) and the 18:3 Area of that Trace (B)

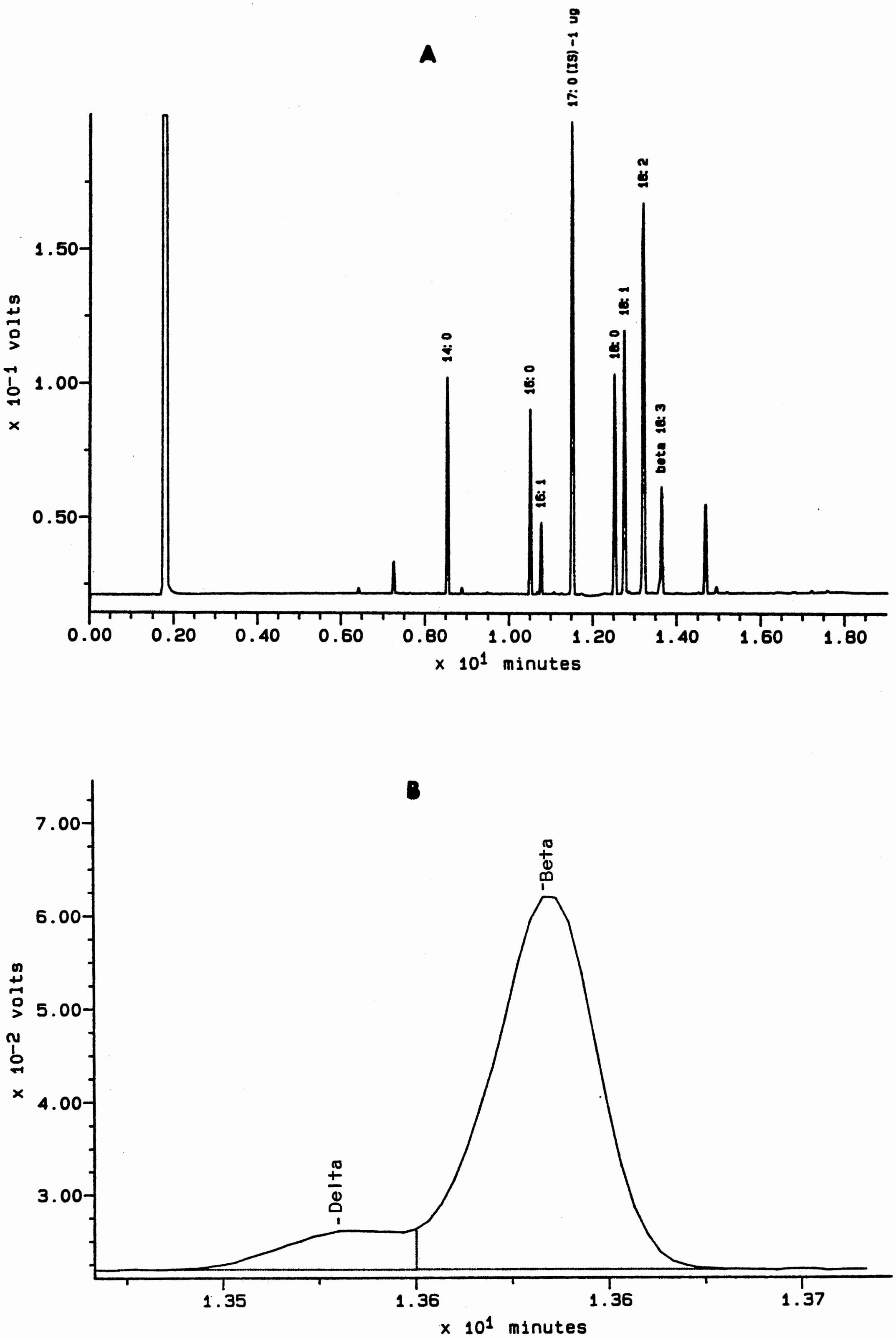


Figure 12. GC Trace of the Phospholipid FAME of the Bird Cherry-Oat Aphid (<u>Rhopalosiphum padi</u>) (A) and the 18:3 Area of that Trace (B)

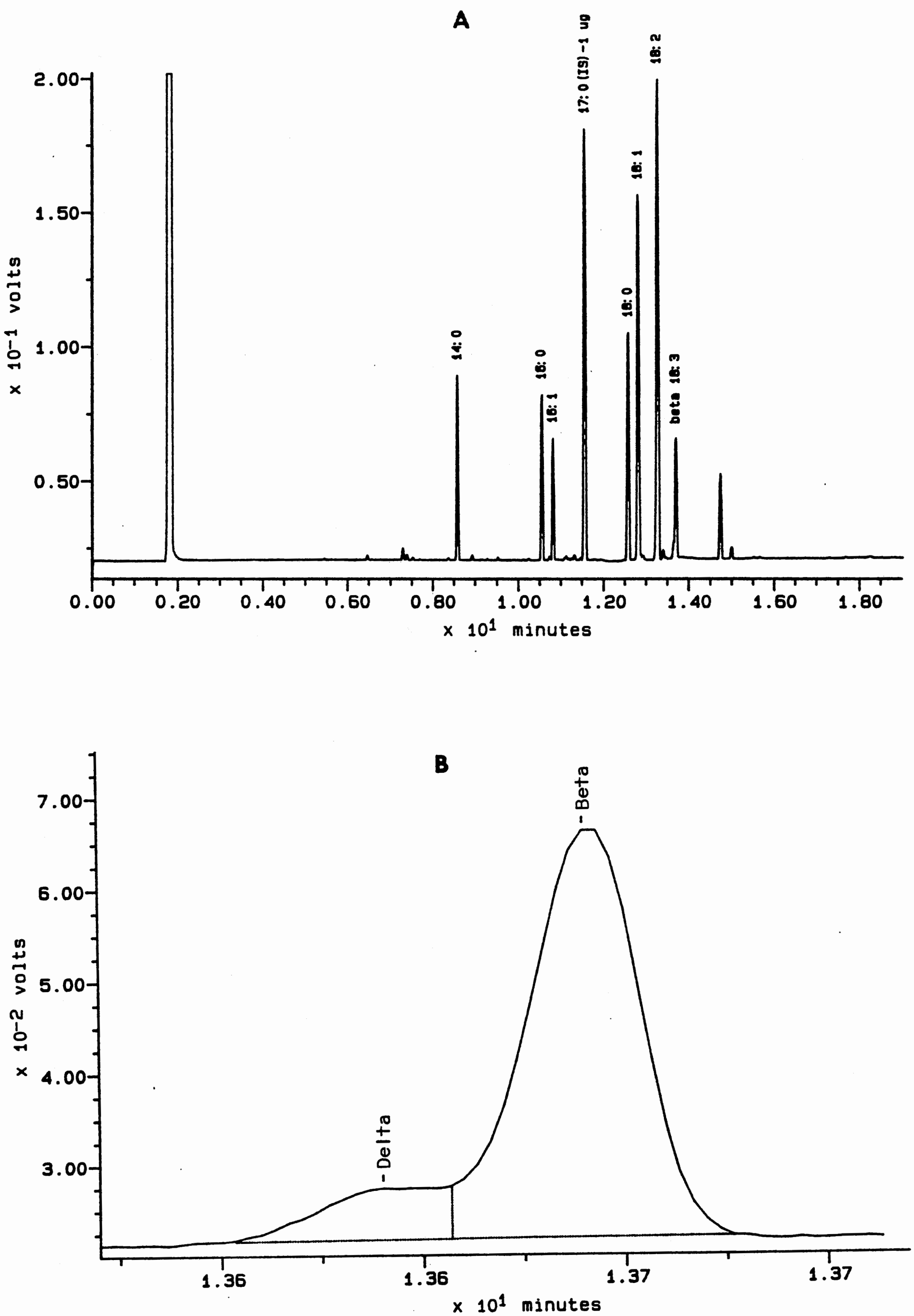


Figure 13. GC Trace of the Phospholipid FAME of the Turnip Aphid (Lipaphis erysimi) (A) and the 18:3 Area of that Trace (B)

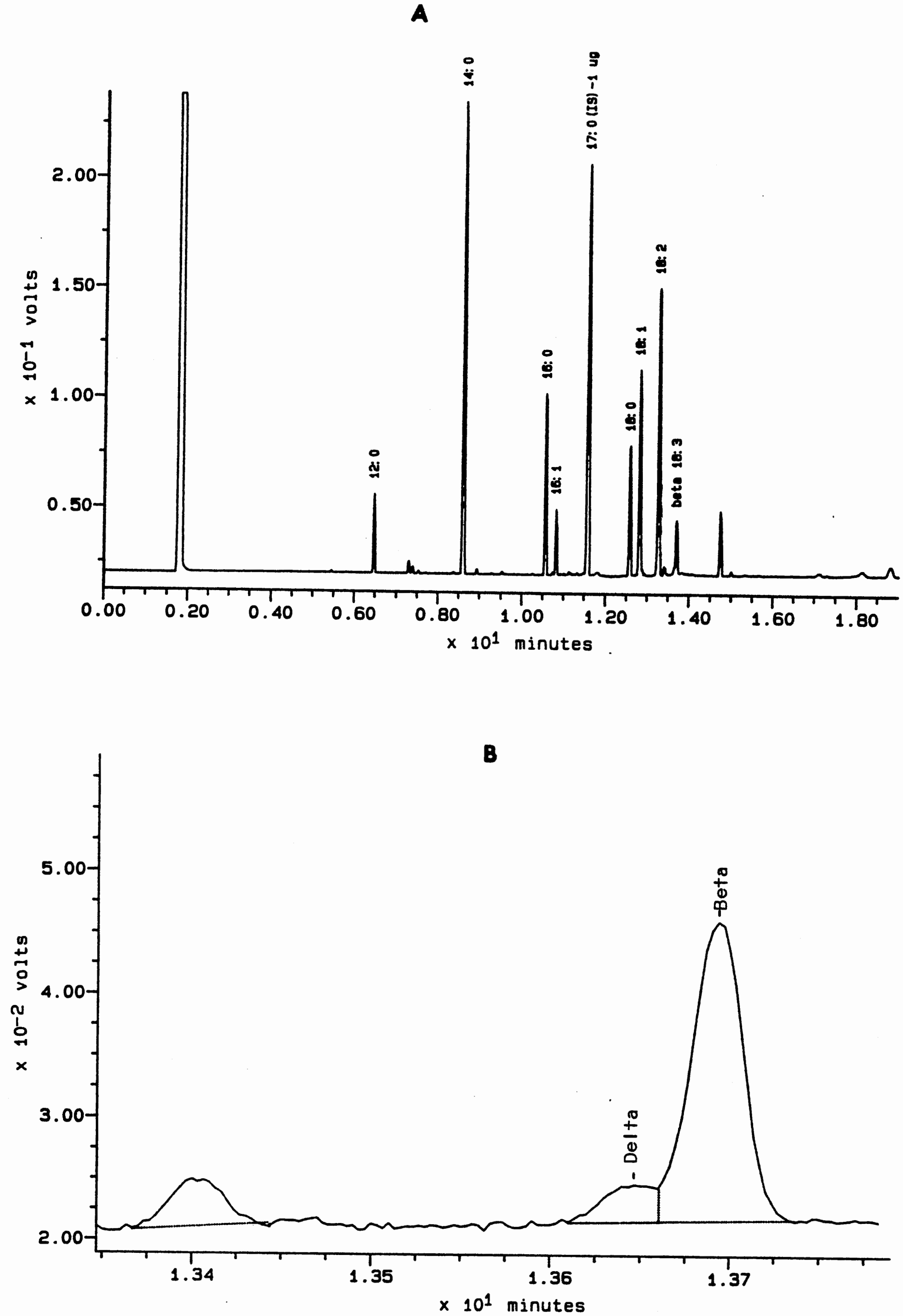
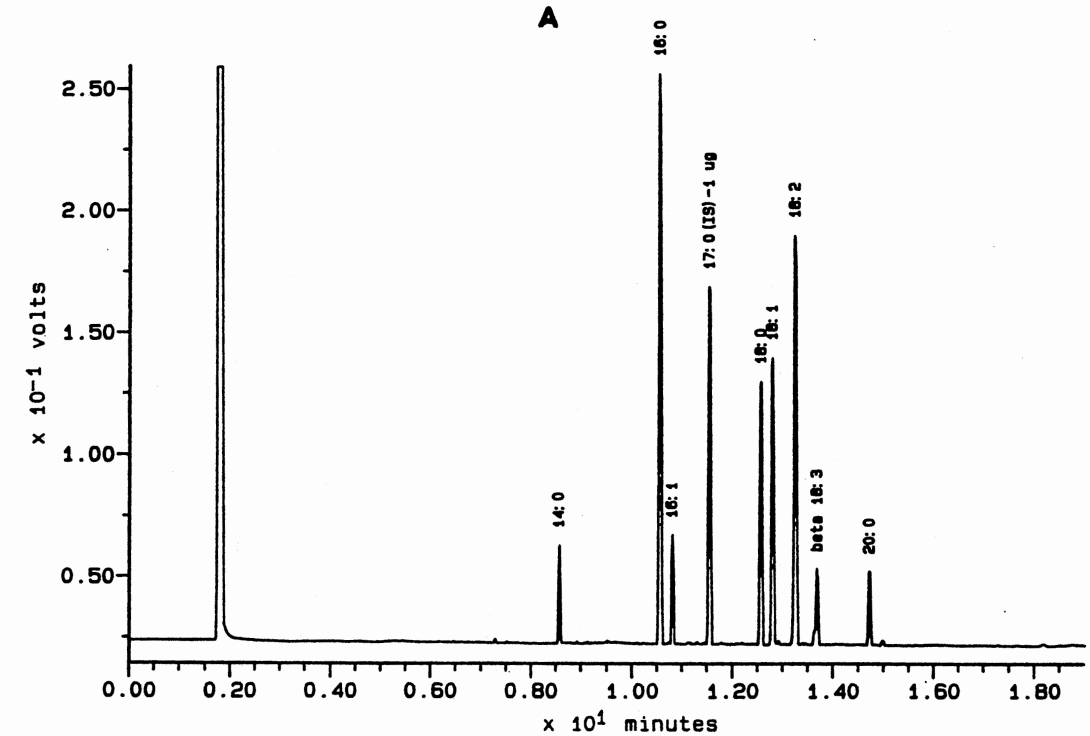


Figure 14. GC Trace of the Phospholipid FAME of the Russian Wheat Aphid (<u>Diuraphis</u> <u>noxia</u>) (A) and the 18:3 Area of that Trace (B)



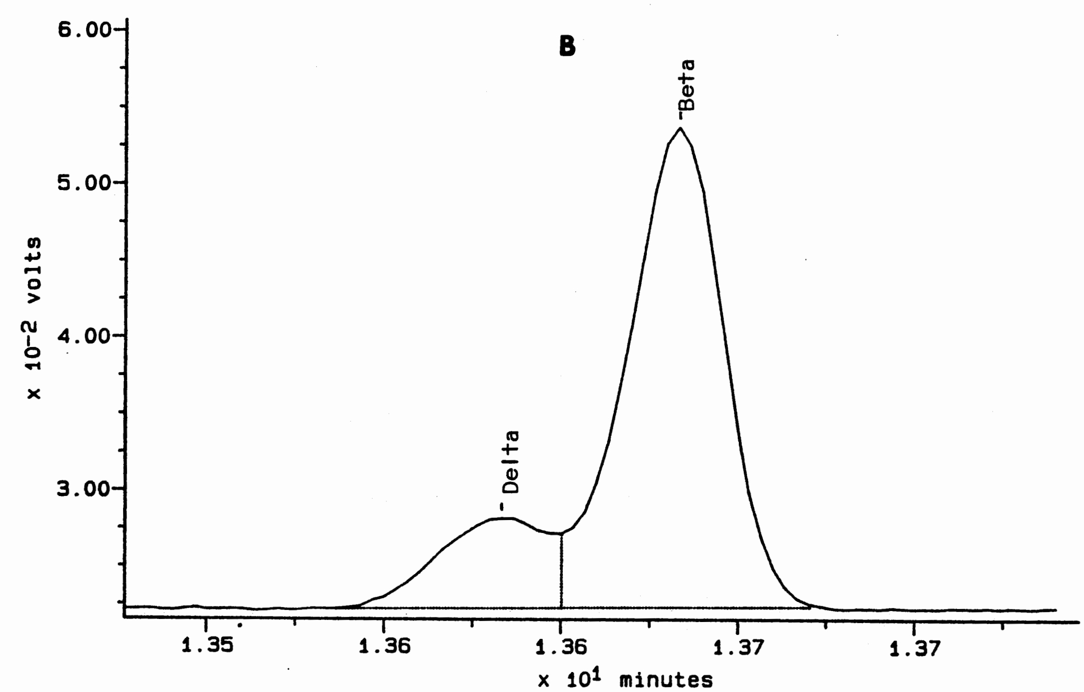


Figure 15. GC Trace of the Phospholipid FAME of the Oleander Aphid (*Aphis nerii*) (A) and the 18:3 Area of that Trace (B)

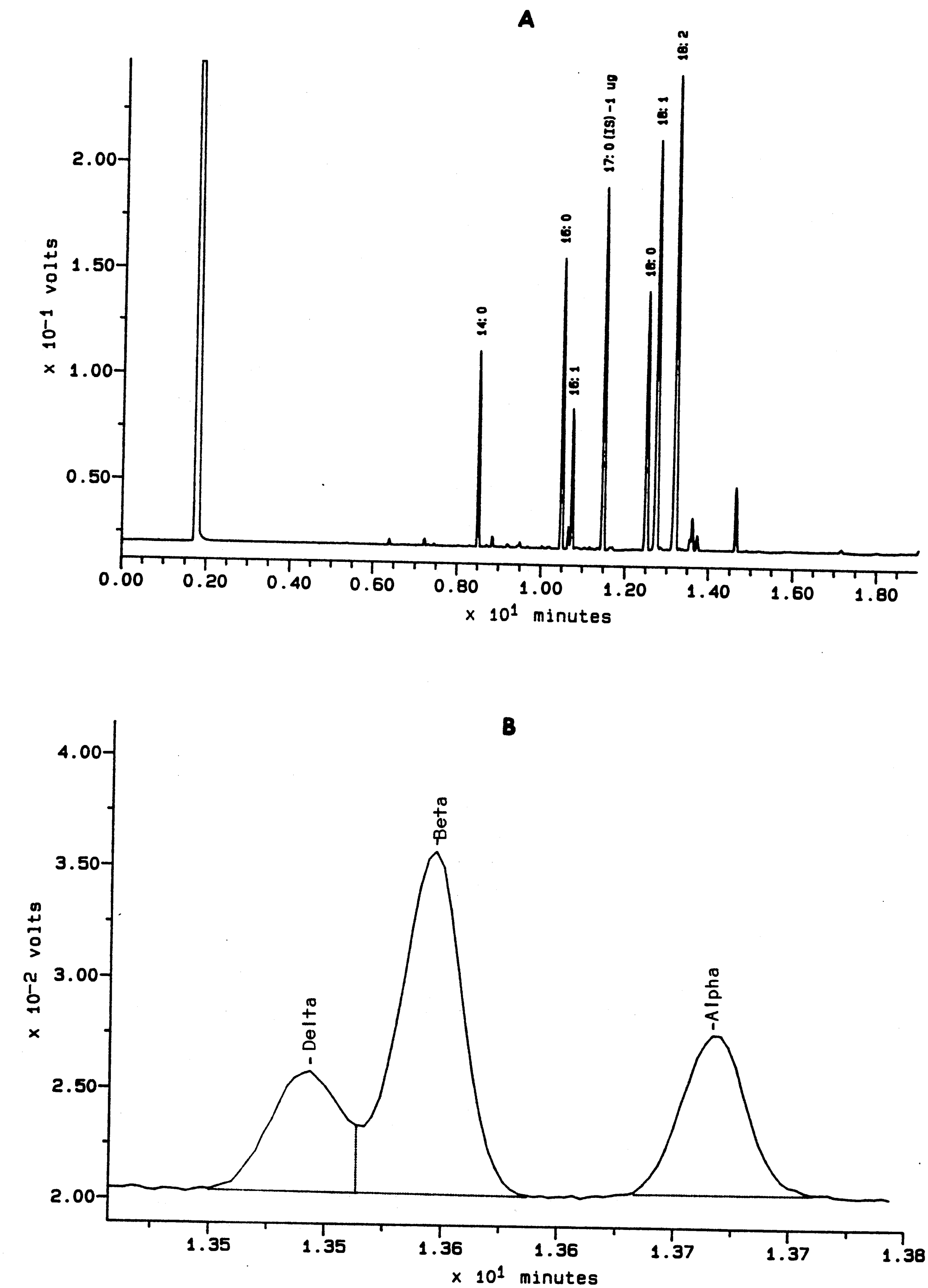
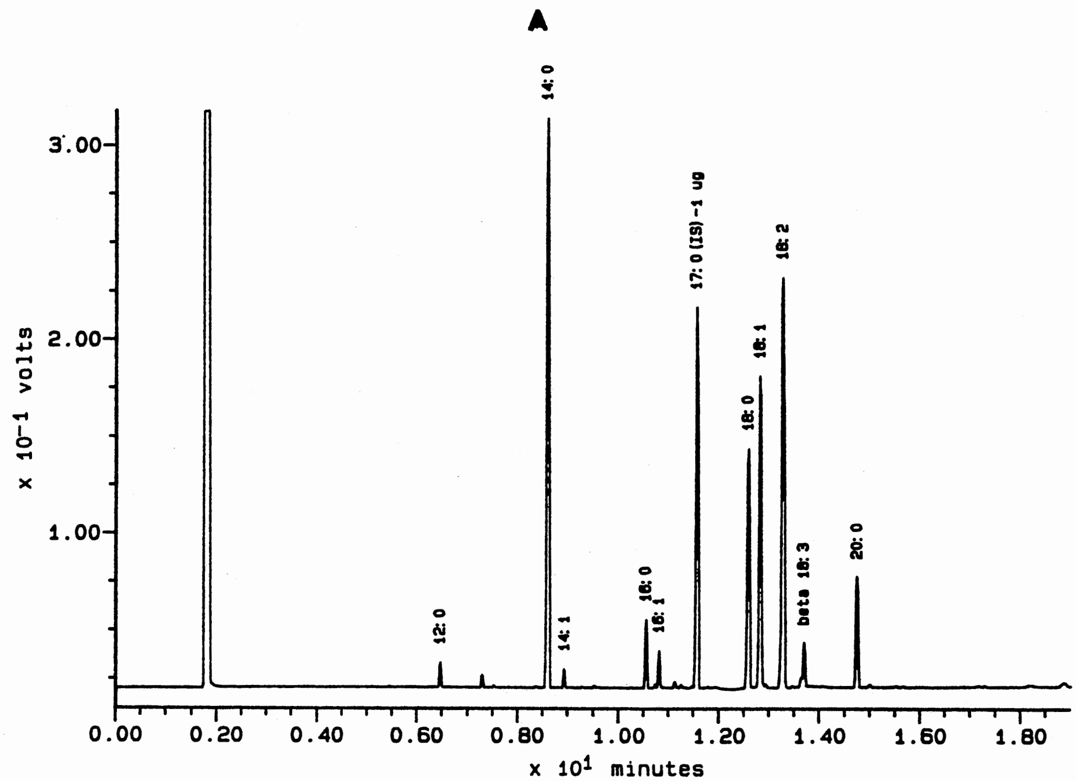


Figure 16. GC Trace of the Phospholipid FAME of the Black Pecan Aphid (<u>Malanocallis caryaefoliae</u>) (A) and the 18:3 Area of that Trace (B)



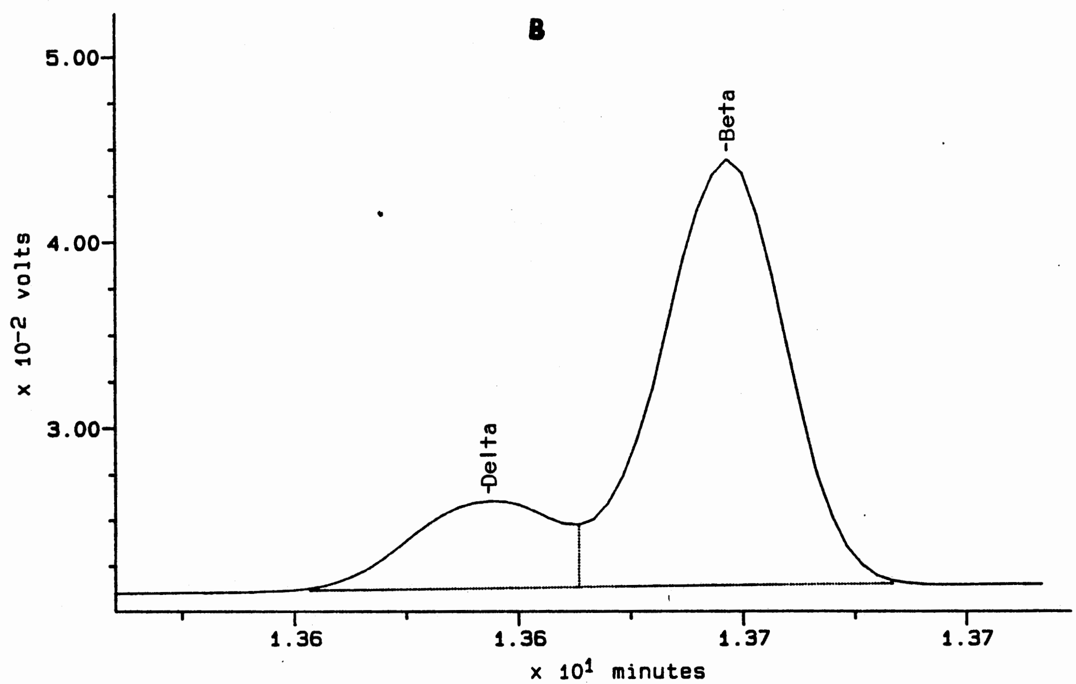


Figure 17. GC Trace of the Phospholipid FAME of the Yellow Sugarcane Aphid (Sipha flava) (A) and the 18:3 Area of that Trace (B)

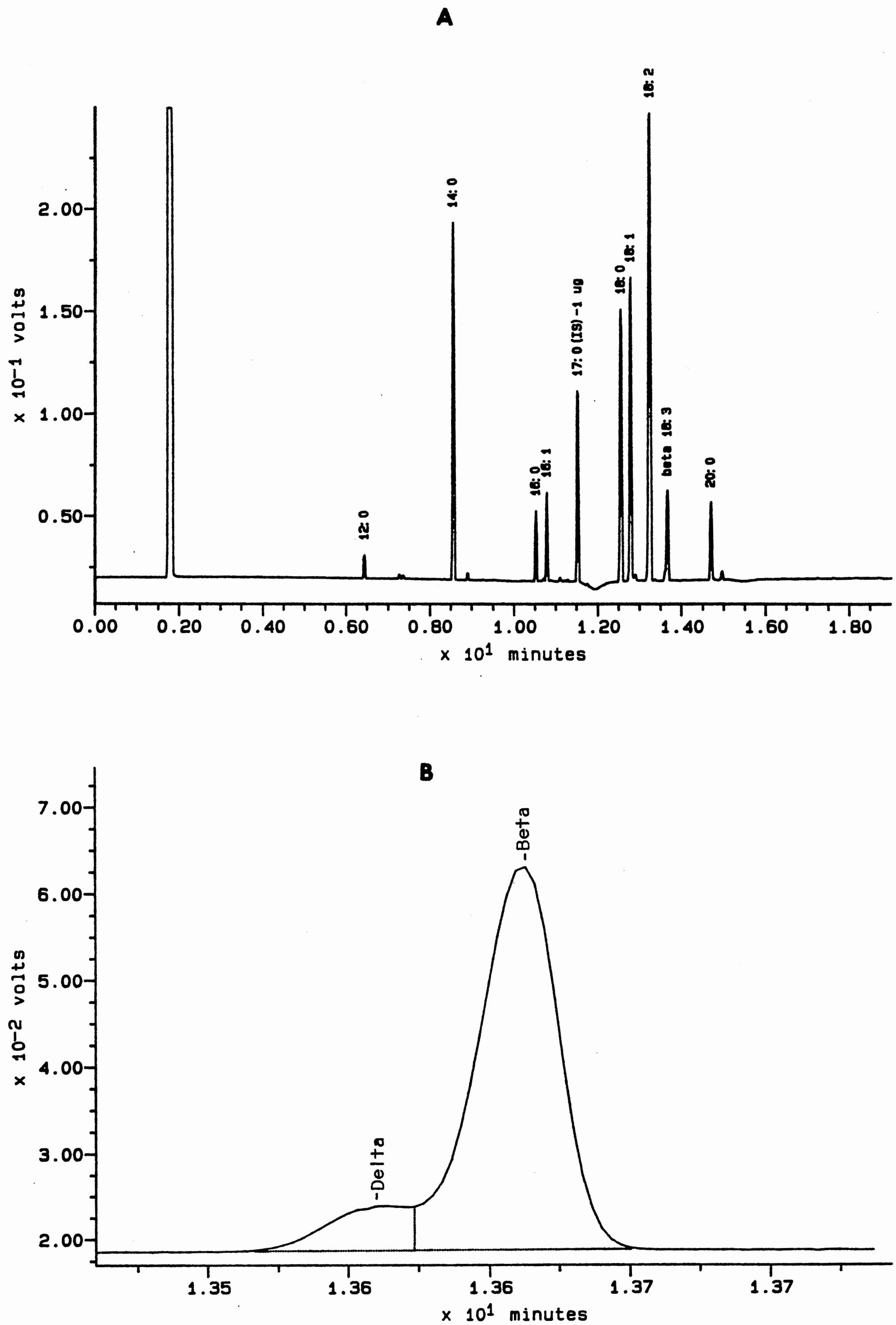


Figure 18. GC Trace of the Phospholipid FAME of the Pea Aphid (Acyrthosiphon pisum) (A) and the 18:3 Area of that Trace (B)

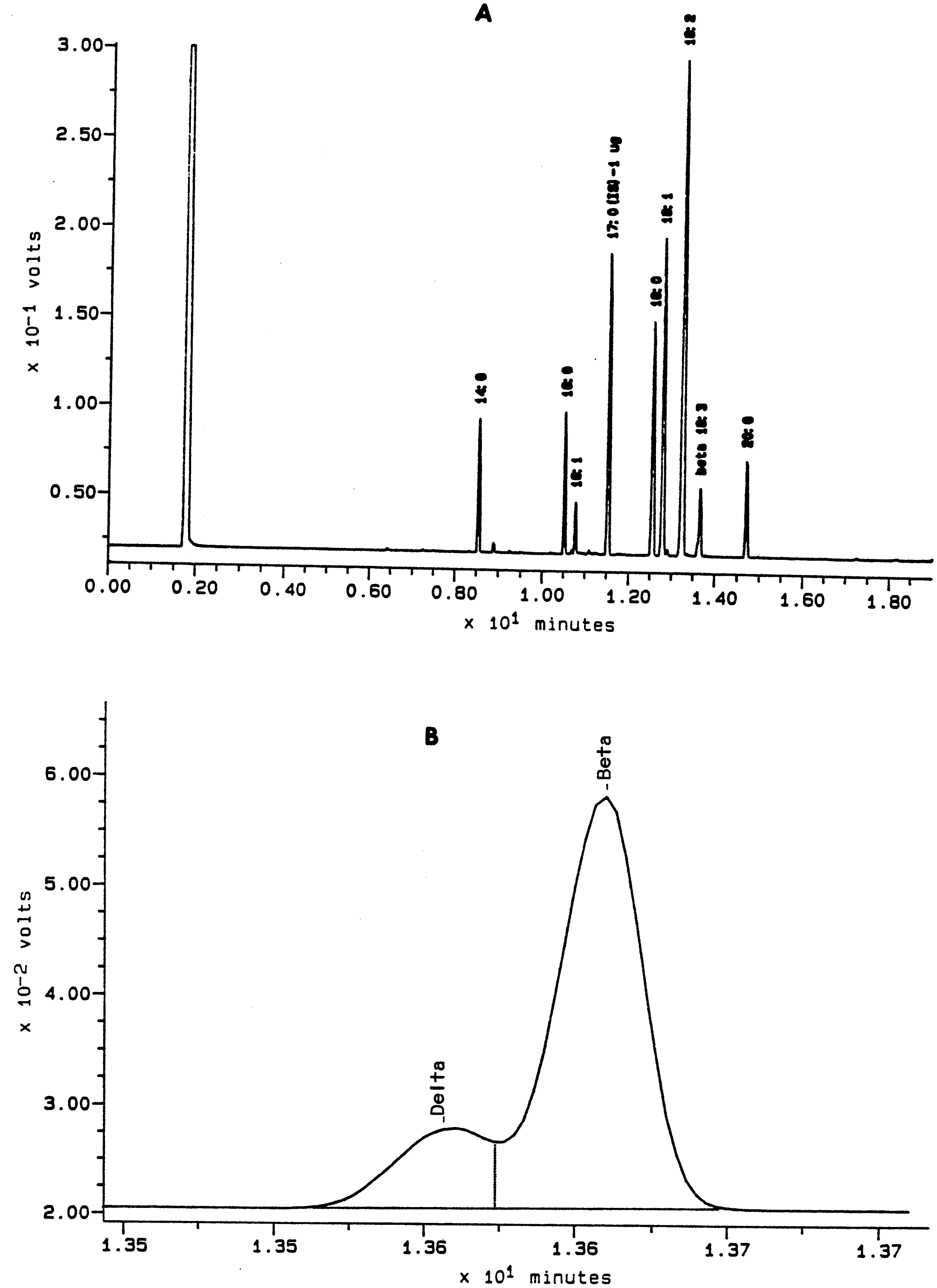


Figure 19. GC Trace of the Phospholipid FAME of the Spotted Alfalfa Aphid (Therioaphis maculata) (A) and the 18:3 Area of that Trace (B)

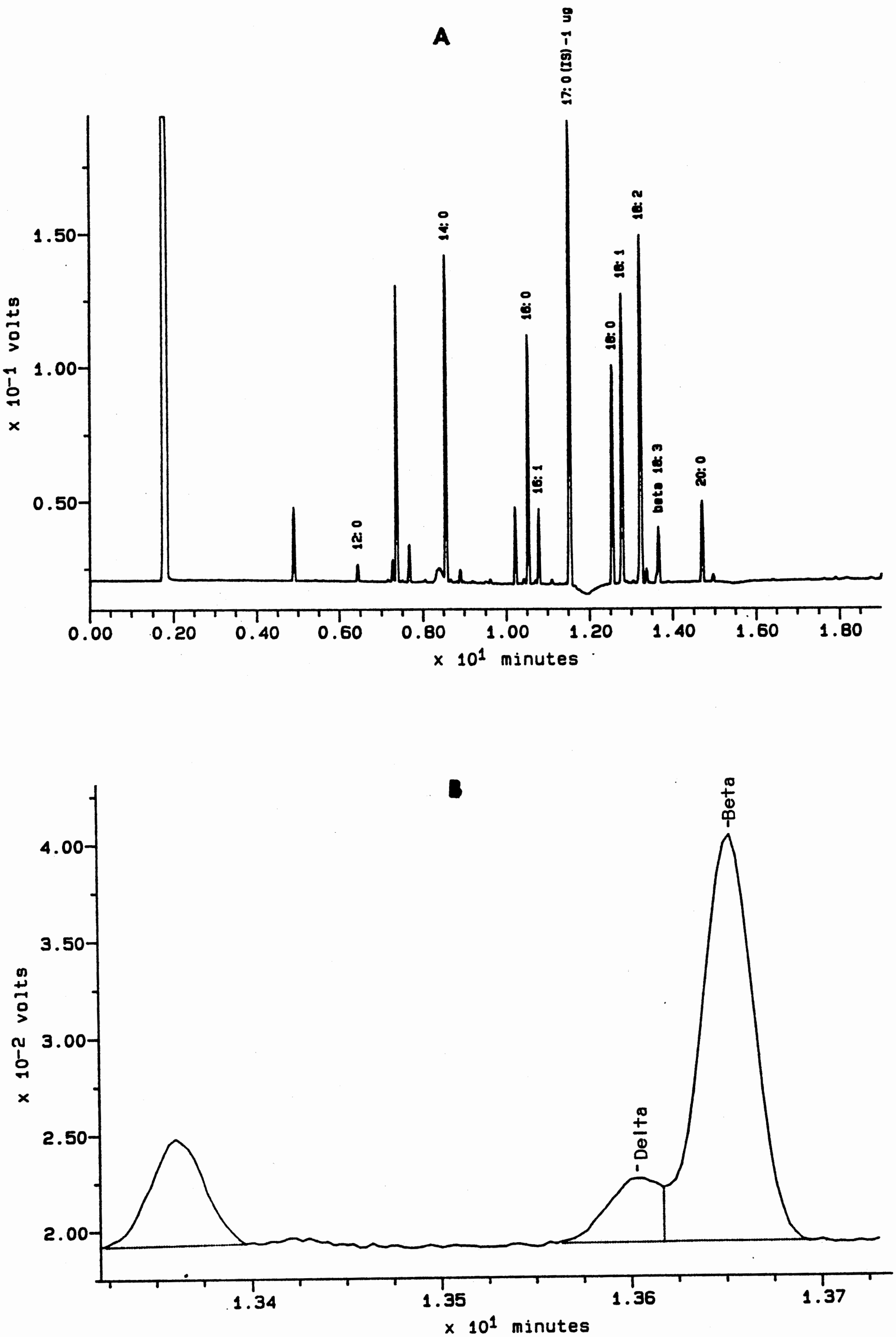


Figure 20. GC Trace of the Phospholipid FAME of the Greenbug (Schizaphis graminum) (A) and the 18:3 Area of that Trace (B)

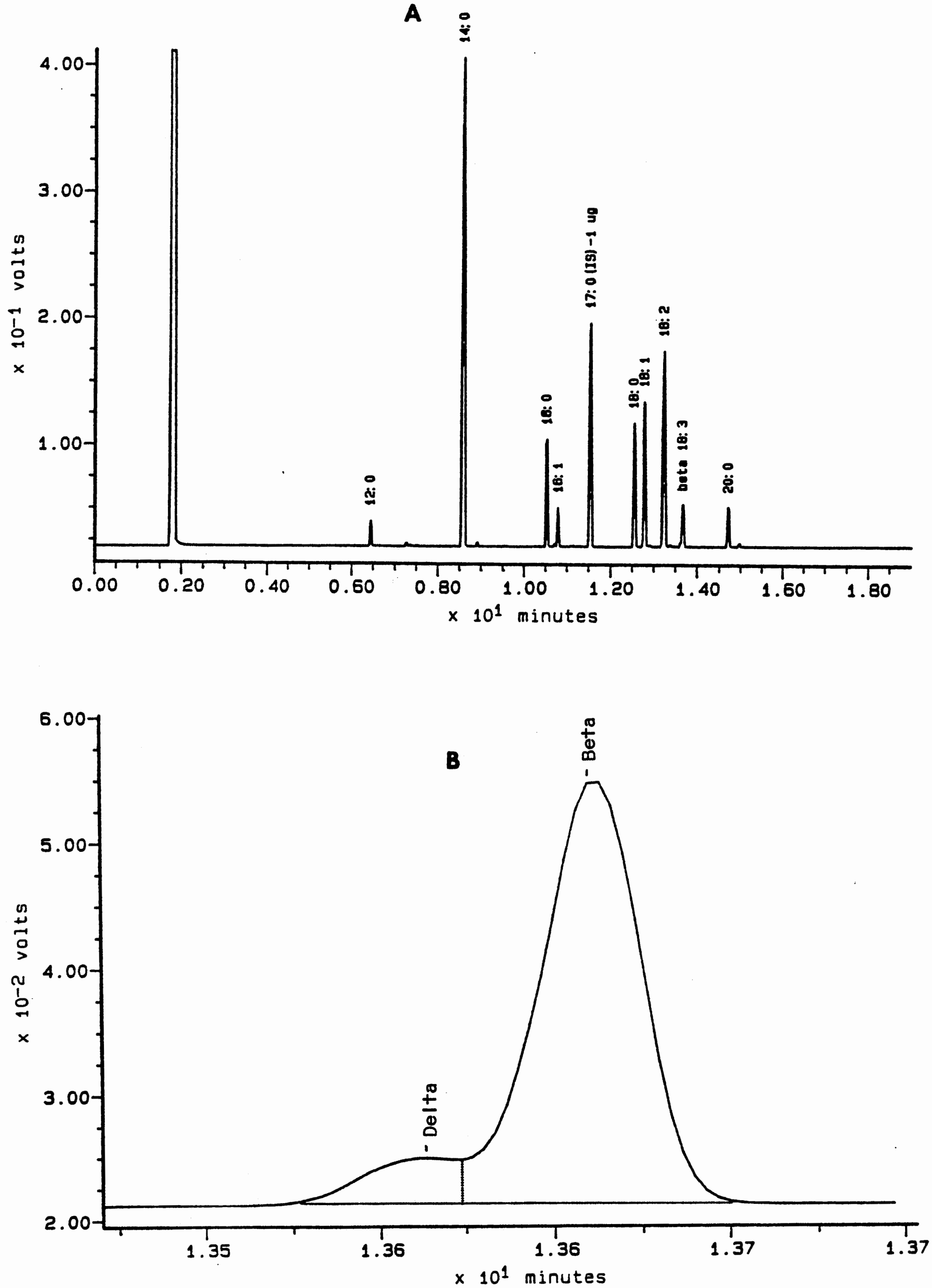


Figure 21. GC Trace of the Phospholipid FAME of the English Grain Aphid (Sitobion avenae) (A) and the 18:3 Area of that Trace (B)

TABLE II

% COMPOSITION OF LINOLENATE ISOMERS IN APHIDS (TOTAL LIPID)

APHIDIDAE	γ18:3	δ18:3	ß18:3	α18:3
Chrysanthemum Aphid	---	13.5 ± 0.3	86.5 ± 0.3	---
Giant Bark Aphid	---	22.1 ± 0.8	77.9 ± 0.8	---
Blue Alfalfa Aphid	---	11.8 ± 0.4	88.2 ± 0.4	---
Birch Aphid	---	21.8 ± 0.4	78.2 ± 0.4	---
Bird Cherry-Oat Aphid	---	10.2 ± 1.1	90.8 ± 1.1	---
Turnip Aphid	---	12.4 ± 1.5	87.6 ± 1.5	---
Russian Wheat Aphid	---	12.3 ± 0.6	87.7 ± 0.6	---
Oleander Aphid	---	17.6 ± 1.2	82.4 ± 1.2	---
Black Pecan Aphid	---	20.9 ± 1.2	45.2 ± 5.8	33.9 ± 7.0
Yellow Sugarcane Aphid	---	17.9 ± 0.8	82.1 ± 0.8	---
Pea Aphid	---	10.2 ± 0.6	89.8 ± 0.6	---
Spotted Alfalfa Aphid	---	20.0	80.0	---
Greenbug	---	14.5 ± 0.6	85.5 ± 0.6	---
English Grain Aphid	---	11.4 ± 2.0	88.6 ± 2.0	---

Note. n = 3, mean ± SD

the linolenic acid was in the phospholipid fraction. The neutral fraction had only traces of linolenic acid in 3 aphids.

% Composition of Fatty Acid in Aphids

Total Fatty Acid Extract

All the aphids in this study lacked capric acid except for blue alfalfa aphids that contained only a trace (Table III). Small portions of lauric acid, 0 to 7.3%, were found, with most aphids having less than 3%. The most abundant fatty acid in aphids was myristic acid with a mean of 40.1%. The exceptions to this were the birch, oleander, and black pecan aphids with only 7.1, 7.8, and 28.0% respectfully. The most abundant fatty acid in the birch and oleander aphids was palmitic acid. The yellow sugarcane aphid was the only aphid to have more than a trace of myristoleic acid. Less than 2.6% of palmitoleic acid was found in all aphids studied. Low percentages of stearic and oleic acids (<15%) were found in all aphids. The β isomer of linolenic acid was the dominant isomer in all aphids. All aphids contained 3% or less of arachidic acid and the chrysanthemum aphid had a trace of arachidonic acid. The giant bark aphid and the blue alfalfa aphid contained a trace of behenic acid.

Neutral Lipid Fraction

In aphids, there was no capric, arachidonic, or behenic acids in the neutral lipid fractions (Table IV). Less than 5% of the fatty acids in most aphids was lauric acid. The myristic acid was dominant in all aphids except the oleander,

TABLE III

% OF EACH FATTY ACID IN THE TOTAL LIPID EXTRACTS FROM APHIDS

	Chrysanthemum Aphid	Giant Bark Aphid	Blue Alfalfa Aphid	Birch Aphid	Bird Cherry-Oat Aphid	Turnip Aphid	Spotted Alfalfa Aphid
10:0	---	---	T	---	---	---	---
12:0	T	3.6 ± 1.4	1.5 ± 0.2	---	0.7 ± 0.3	1.1 ± 0.1	1.2 ± 0.3
14:0	32.1 ± 2.3	34.5 ± 4.3	60.0 ± 2.1	7.1 ± 3.4	32.1 ± 9.3	37.3 ± 1.8	29.2 ± 1.3
14:1	---	T	T	---	T	---	---
16:0	22.2 ± 2.2	6.9 ± 0.8	4.2 ± 0.1	41.7 ± 2.0	25.4 ± 1.9	26.3 ± 7.2	20.3 ± 0.5
16:1	2.6 ± 0.8	1.9 ± 0.3	1.3 ± 0.1	1.5 ± 0.2	2.1 ± 0.5	2.0 ± 0.6	1.2 ± 0.1
18:0	8.2 ± 0.6	9.5 ± 0.6	6.0 ± 0.2	13.1 ± 0.3	7.8 ± 1.6	6.8 ± 0.2	11.9 ± 0.5
18:1	11.9 ± 0.7	14.8 ± 1.5	9.7 ± 0.7	11.7 ± 1.5	10.0 ± 2.6	8.9 ± 2.5	12.2 ± 0.3
18:2	19.1 ± 1.5	23.3 ± 3.3	13.2 ± 1.0	20.4 ± 1.9	15.6 ± 4.5	12.9 ± 3.5	19.9 ± 0.4
γ18:3	---	---	---	---	---	---	---
δ18:3	0.4	0.5 ± 0.1	0.3	0.5	0.4 ± 0.1	0.3 ± 0.1	0.4
ß18:3	2.3 ± 0.2	1.8 ± 0.3	1.9 ± 0.2	1.9 ± 0.1	3.2 ± 0.8	2.2 ± 1.0	1.6 ± 0.1
α18:3	---	---	---	---	---	---	---
20:0	3.0 ± 0.1	2.6 ± 0.3	2.0 ± 0.1	2.0 ± 0.2	2.5 ± 0.2	1.4 ± 0.5	2.5 ± 0.1
20:4	T	---	---	---	---	---	---
22:0	---	T	T	---	---	---	---

TABLE III (Continued)

	Russian Wheat Aphid	Oleander Aphid	Black Pecan Aphid	Yellow Sugarcane Aphid	Greenbug (Biotype E)	Pea Aphid	English Grain Aphid
10:0	---	---	---	---	---	---	---
12:0	7.3 ± 0.2	0.1 ± 0.1	T	1.1 ± 0.1	2.3 ± 0.1	3.8 ± 0.1	3.0 ± 0.1
14:0	52.7 ± 0.5	7.8 ± 1.4	28.0 ± 1.5	65.4 ± 1.2	46.9 ± 0.4	56.7 ± 0.8	71.2 ± 3.0
14:1	---	---	---	0.2 ± 0.1	T	---	---
16:0	14.1 ± 1.5	66.3 ± 0.9	34.2 ± 1.3	3.5 ± 0.2	31.9	3.5 ± 0.1	5.4 ± 2.3
16:1	1.5 ± 0.1	1.5 ± 0.2	2.0 ± 0.2	0.6	1.4	1.9 ± 0.1	1.1 ± 0.1
18:0	4.9 ± 0.3	5.0 ± 0.1	10.5 ± 0.3	5.6 ± 0.3	6.3 ± 0.1	6.3 ± 0.1	3.5 ± 0.5
18:1	6.5 ± 0.3	7.0 ± 0.9	10.6 ± 1.3	8.3 ± 0.3	7.0 ± 0.1	9.1 ± 0.2	4.1 ± 1.5
18:2	10.3 ± 0.1	10.2 ± 1.2	11.8 ± 0.8	12.4 ± 0.6	8.7 ± 0.2	16.6 ± 0.5	7.8 ± 0.4
γ18:3	---	---	---	---	---	---	---
δ18:3	0.2	0.2	0.3	0.2	0.2	0.2	0.1 ± 0.1
ß18:3	1.5 ± 0.1	0.9 ± 0.1	0.6 ± 0.1	0.9 ± 0.1	1.1 ± 0.1	2.3 ± 0.1	1.5 ± 0.2
α18:3	---	---	0.4 ± 0.1	---	---	---	---
20:0	1.6 ± 0.1	0.9	1.3 ± 0.1	1.8 ± 0.1	1.3	1.4	1.0 ± 0.1
20:4	---	---	---	---	---	---	---
22:0	---	---	---	---	---	---	---

Note: n = 3, means ± SD, T = >0.05.

TABLE IV

% OF EACH FATTY ACID IN THE NEUTRAL LIPID FRACTION FROM APHIDS

	Giant Bark Aphid	Blue Alfalfa Aphid	Birch Aphid	Bird Cherry-Oat Aphid	Turnip Aphid	Russian Wheat Aphid	Oleander Aphid
12:0	1.3 ± 1.6	1.8 ± 0.5	---	1.2 ± 0.3	1.5	8.8 ± 0.8	T
14:0	61.9 ± 3.4	89.6 ± 1.8	20.7 ± 13.0	47.6 ± 5.6	51.5 ± 5.2	66.1 ± 1.8	9.2 ± 1.5
14:1	---	---	---	T	---	---	---
16:0	13.9 ± 0.9	5.3 ± 0.1	63.6 ± 12.1	37.0 ± 4.3	39.4 ± 5.1	17.3 ± 0.1	80.0 ± 0.5
16:1	---	T	T	0.7 ± 0.2	---	0.3 ± 0.3	0.5 ± 0.2
18:0	6.9 ± 0.3	2.2 ± 0.2	7.9 ± 0.5	4.1 ± 0.6	5.0 ± 0.5	2.8 ± 0.2	2.8 ± 0.1
18:1	6.8 ± 1.3	2.1 ± 0.2	4.0 ± 0.8	4.2 ± 0.9	1.4 ± 0.3	2.3 ± 0.9	3.9 ± 1.0
18:2	3.6 ± 0.9	1.1 ± 0.2	4.4 ± 0.6	4.0 ± 0.	1.1 ± 0.6	2.2 ± 1.2	3.5 ± 0.9
γ18:3	---	---	---	---	---	---	---
δ18:3	---	---	T	T	---	T	---
β18:3	---	---	T	T	---	0.2 ± 0.2	---
α18:3	---	---	---	---	---	---	---
20:0	2.3 ± 0.6	0.6	T	0.6 ± 0.2	T	0.3 ± 0.2	0.1 ± 0.1

TABLE IV (Continued)

	Black Pecan Aphid	Yellow Sugarcane Aphid	Greenbug (Biotype E)	Pea Aphid	Spotted Alfalfa Aphid	English Grain Aphid
12:0	1.1 ± 0.03	2.5 ± 0.1	2.9 ± 0.2	5.4 ± 0.5	1.3 ± 0.7	3.2 ± 0.3
14:0	39.8 ± 2.7	89.9 ± 0.7	44.4 ± 0.4	86.2 ± 0.3	45.7 ± 1.9	87.4 ± 2.0
14:1	---	---	---	---	---	---
16:0	43.7 ± 0.3	3.8 ± 0.3	36.7 ± 0.6	3.9 ± 0.1	33.2 ± 1.2	5.7 ± 4.3
16:1	0.5 ± 0.1	---	---	---	0.2 ± 0.2	T
18:0	7.4 ± 0.5	1.8 ± 0.3	4.2 ± 0.2	1.9 ± 0.1	10.1 ± 0.7	1.4 ± 0.4
18:1	4.2 ± 1.5	0.6 ± 0.1	1.3 ± 0.1	1.7	6.0 ± 0.3	1.2 ± 0.2
18:2	2.7 ± 0.3	0.5 ± 0.1	0.6	1.1 ± 0.1	3.0 ± 0.3	0.4 ± 0.4
γ18:3	---	---	---	---	---	---
δ18:3	---	---	---	---	---	---
β18:3	---	---	---	---	---	T
α18:0	0.4 ± 0.2	---	---	---	---	---
20:0	0.3	0.2 ± 0.1	0.3	0.2	0.5	T

Note: n = 3, means ± SD, T = >0.05.

birch, and black pecan aphid where palmitic acid was dominant. Stearic, oleic, and linoleic acid occurred in low percentages in neutral lipids. The neutral lipid generally lacked all linolenic acid isomers, with only a few aphids having traces of β and δ isomers. The black pecan aphid contained 0.4% of α-linolenic acid. All aphids showed a trace of arachidic acid which was generally less than 0.5%.

Phospholipid Fraction

As with the neutral lipid, capric, arachidonic and behenic acids were absent in the PL fraction (Table V). Lauric acid was present at low percentages in one half of the aphids studied (giant bark, Russian wheat, yellow sugarcane, greenbug, pea, and English grain aphids). Percentage of myristic and palmitic acids were low when compared to the total and neutral lipid samples. There was more palmitoleic acid in the phospholipid than neutral lipid fraction. Stearic, oleic, linoleic acids occurred in greater proportion. As with the total lipid, the phospholipid fraction contained both the β and δ-linolenic acid isomers. Again, the PL fraction of black pecan aphids also contained the α-isomer. The γ-isomer was not present in all samples. Most of the arachidic was present in the phospholipid fraction.

μg of Each Fatty Acid/mg Aphid

Total Lipid Extract

In general, Table VI, containing μg of fatty acid per mg aphid, reflected its counterpart, Table III, that contains percentages of fatty acids. There was a large

TABLE V

% OF EACH FATTY ACID IN THE PHOSPHOLIPID FRACTION FROM APHIDS

	Giant Bark Aphid	Blue Alfalfa Aphid	Birch Aphid	Bird Cherry-Oat Aphid	Turnip Aphid	Russian Wheat Aphid	Oleander Aphid
12:0	0.7 ± 0.8	---	---	---	---	3.0 ± 1.6	---
14:0	9.6 ± 4.0	14.7 ± 7.7	4.4 ± 3.7	7.2 ± 3.8	16.1 ± 8.8	22.2 ± 6.4	2.9 ± 0.9
14:1	0.4 ± 0.4	---	---	---	---	---	---
16:0	3.9 ± 0.4	3.3 ± 0.5	12.7 ± 1.9	5.9 ± 1.0	15.7 ± 8.6	8.0 ± 1.6	27.2 ± 5.3
16:1	2.9 ± 0.3	3.5 ± 0.4	2.5 ± 0.5	4.2 ± 0.3	4.1 ± 1.1	3.7 ± 0.3	4.6 ± 0.5
18:0	15.4 ± 1.4	14.9 ± 2.7	17.8 ± 1.0	16.1 ± 1.0	12.8 ± 2.0	10.5 ± 1.8	13.2 ± 0.8
18:1	21.8 ± 1.2	21.8 ± 1.8	18.5 ± 2.5	18.9 ± 1.1	16.1 ± 5.1	15.5 ± 2.2	15.8 ± 1.5
18:2	37.5 ± 2.8	32.0 ± 2.6	35.5 ± 2.3	33.1 ± 1.9	26.0 ± 7.5	26.5 ± 4.2	28.8 ± 3.8
γ18:3[1]	---	---	---	---	---	---	---
δ18:3[1]	1.0 ± 0.1	0.6 ± 0.1	1.0 ± 0.1	0.9	0.6 ± 0.2	0.5 ± 0.2	0.7 ± 0.1
ß18:3[1]	3.0 ± 0.3	4.7 ± 0.3	4.2 ± 0.8	7.4 ± 0.2	4.1 ± 2.0	4.3 ± 1.0	3.4 ± 0.3
α18:3[1]	---	---	---	---	---	---	---
20:0	3.9 ± 0.6	4.7 ± 0.8	3.4 ± 0.3	6.4 ± 0.5	4.1 ± 0.8	5.2 ± 1.0	3.6 ± 0.2

TABLE V (Continued)

	Black Pecan Aphid	Yellow Sugarcane Aphid	Greenbug (Biotype E)	Pea Aphid	Spotted Alfalfa Aphid	English Grain Aphid
12:0	---	0.4 ± 0.4	0.8 ± 0.1	0.8 ± 0.3	---	1.2 ± 1.1
14:0	11.2 ± 3.4	20.5 ± 8.5	16.5 ± 2.8	16.0 ± 2.9	5.8 ± 1.2	37.9 ± 9.5
14:1	---	0.5 ± 0.4	---	T	---	---
16:0	15.2 ± 2.5	2.4 ± 1.9	13.3 ± 1.8	2.9 ± 0.1	6.1 ± 0.7	4.7 ± 1.7
16:1	4.7 ± 0.5	1.6 ± 1.5	3.8 ± 0.2	3.7 ± 0.1	2.1	2.8 ± 0.5
18:0	16.0 ± 1.0	13.5 ± 0.7	12.4 ± 1.3	14.8 ± 1.6	17.1 ± 1.4	9.8 ± 2.0
18:1	20.5 ± 2.7	20.7 ± 3.6	18.8 ± 1.4	18.1 ± 1.2	19.7 ± 1.2	14.3 ± 3.1
18:2	26.6 ± 2.7	32.1 ± 4.9	25.4 ± 2.1	34.7 ± 2.0	39.4 ± 1.5	21.6 ± 4.8
γ18:3[1]	---	---	---	---	---	---
δ18:3[1]	0.5	0.6 ± 0.1	0.5 ± 0.1	0.5	0.8 ± 0.1	0.4 ± 0.1
β18:3[1]	1.4 ± 0.1	2.1 ± 0.3	3.5 ± 0.3	4.9 ± 0.3	3.6 ± 0.1	4.3 ± 1.0
α18:3[1]	0.5 ± 0.2	---	---	---	---	---
20:0	3.1 ± 0.2	5.5 ± 0.5	4.4 ± 0.6	3.6 ± 0.4	5.9 ± 0.4	3.3 ± 0.7

[1] Methyl esters of linolenic acid isomers confirmed by GC-MS. Note: n = 3, means ± SD, T = >0.05.

TABLE VI

μg OF EACH FATTY ACID IN THE TOTAL LIPID EXTRACT/mg APHID

	Chrysanthemum Aphid	Giant Bark Aphid	Blue Alfalfa Aphid	Birch Aphid	Bird Cherry-Oat Aphid	Turnip Aphid	Spotted Alfalfa Aphid
10:0	---	---	---	---	---	---	---
12:0	T	1.1 ± 0.5	0.4 ± 0.3	---	0.2 ± 0.2	0.4	0.3 ± 0.1
14:0	7.4 ± 1.4	10.4 ± 2.5	16.8 ± 2.2	3.8 ± 2.1	9.9 ± 6.5	17.4 ± 3.3	7.5 ± 0.7
14:1	---	---	T	---	T	---	---
16:0	5.2 ± 1.1	2.9 ± 0.4	1.2 ± 0.2	21.8 ± 3.2	7.4 ± 3.2	13.7 ± 0.5	5.2 ± 0.2
16:1	0.5	0.6	0.4 ± 0.1	0.8 ± 0.1	0.6 ± 0.1	0.7 ± 0.1	0.3
18:0	1.9	2.8 ± 0.2	1.7 ± 0.3	6.9 ± 0.8	2.1 ± 0.1	2.8 ± 0.1	3.0
18:1	0.8 ± 0.1	4.4 ± 0.2	2.7 ± 0.6	6.1 ± 0.2	2.7 ± 0.2	2.9 ± 0.7	3.1 ± 0.1
18:2	4.5 ± 0.2	6.9 ± 0.1	3.7 ± 0.8	10.4 ± 0.4	4.2 ± 0.1	4.9 ± 0.9	5.1 ± 0.2
γ 18:3	---	---	---	---	---	---	---
δ18:3	0.1	0.2	0.1	0.3	0.1	0.1	0.1
ß18:3	0.6 ± 0.2	0.5	0.5 ± 0.1	1.0	0.9 ± 0.1	0.7 ± 0.3	0.4
α18:3	---	---	---	---	---	---	---
20:0	0.7	0.8	0.6 ± 0.1	1.0	0.7 ± 0.1	0.7 ± 0.1	0.6 ± 0.1
20:4	---	---	---	---	---	---	---
22:0	---	T	---	T	---	---	---
Total	23.7 ± 2.9	31.0 ± 4.7	28.3 ± 4.3	52.4 ± 5.6	28.1 ± 10.8	39.0 ± 4.1	25.6 ± 1.3

TABLE VI (Continued)

	Russian Wheat Aphid	Oleander Aphid	Black Pecan Aphid	Yellow Sugarcane Aphid	Greenbug (Biotype E)	Pea Aphid	English Grain Aphid
10:0	---	T	---	---	---	---	---
12:0	2.7 ± 0.4	0.1	T	0.4	1.0 ± 0.1	0.7	1.7 ± 0.2
14:0	21.1 ± 1.0	3.9 ± 1.1	11.9 ± 1.2	24.2 ± 0.6	21.2 ± 1.6	10.8 ± 0.4	39.5 ± 5.4
14:1	---	---	---	T	---	---	---
16:0	5.3	33.0 ± 4.4	14.6 ± 2.0	1.3 ± 0.1	14.4 ± 1.0	0.7	2.9 ± 1.0
16:1	0.6 ± 0.2	0.8 ± 0.1	0.8	0.2	0.6	0.4	0.6
18:0	1.9 ± 0.1	2.5 ± 0.3	4.5 ± 0.6	2.1 ± 0.1	2.9 ± 0.1	1.2	1.9 ± 0.1
18:1	2.6 ± 0.2	3.5 ± 0.5	4.5 ± 0.8	3.1 ± 0.1	3.1 ± 0.2	1.7 ± 0.1	3.1 ± 0.4
18:2	4.3 ± 0.2	5.1 ± 0.8	5.0 ± 0.4	4.6 ± 0.2	3.9 ± 1.0	3.2 ± 0.1	4.3 ± 0.4
γ18:3	---	---	---	---	---	---	---
δ18:3	0.1	0.1	0.1	0.1	0.1	0.1	0.1
β18:3	0.6	0.5 ± 0.1	0.2	0.3	0.5	0.4	0.8 ± 0.2
α18:3	---	---	0.2 ± 0.7	---	---	---	---
20:0	0.7	0.5 ± 0.1	0.6 ± 0.1	0.7	0.6	0.3	0.7 ± 0.3
20:4	---	---	---	---	---	---	---
22:0	---	---	---	---	---	---	---
Total	40.2 ± 1.5	49.9 ± 6.5	42.8 ± 4.6	37.2 ± 0.6	48.2 ± 3.2	19.3 ± 0.5	55.4 ± 5.4

Note: n = 3, means ± SD, T = >0.05.

TABLE VII

μg OF EACH FATTY ACID IN THE NEUTRAL LIPID FRACTION/mg APHID

	Giant Bark Aphid	Blue Alfalfa Aphid	Birch Aphid	Bird Cherry-Oat Aphid	Turnip Aphid	Russian Wheat Aphid	Oleander Aphid
12:0	0.2 ± 0.1	0.3 ± 0.1	---	0.2 ± 0.2	0.5 ± 0.1	2.4 ± 0.3	T
14:0	5.7 ± 2.0	13.9 ± 2.0	3.3 ± 2.9	8.0 ± 5.6	14.3 ± 1.8	18.2 ± 3.3	3.3 ± 0.7
14:1	---	---	---	T	---	---	---
16:0	1.3 ± 0.4	0.8 ± 0.2	9.0 ± 2.5	5.7 ± 2.8	11.2 ± 3.5	4.8 ± 1.0	29.1 ± 2.0
16:1	---	---	T	0.1 ± 0.1	---	0.2 ± 0.3	0.2 ± 0.1
18:0	0.6 ± 0.2	0.3 ± 0.1	1.2 ± 0.4	0.6 ± 0.3	1.4 ± 0.4	0.8 ± 0.2	1.0
18:1	0.6 ± 0.2	0.3 ± 0.1	0.6 ± 0.2	0.6 ± 0.2	0.4 ± 0.1	0.7 ± 0.3	1.4 ± 0.4
18:2	0.3 ± 0.4	0.2 ± 0.1	0.6 ± 0.1	0.6 ± 0.2	0.4 ± 0.1	0.7 ± 0.4	1.3 ± 0.4
γ18:3	---	---	---	---	---	---	---
δ18:3	---	---	---	T	---	T	---
β18:3	---	---	---	T	---	0.1 ± 0.1	---
α18:3	---	---	---	---	---	---	---
20:0	0.2	0.1	T	0.1	T	0.1 ± 0.1	0.2 ± 0.1
22:0	0.3	---	---	---	---	---	---
Total	9.2 ± 2.8	15.9 ± 2.3	14.8 ± 5.3	16.1 ± 9.8	28.0 ± 5.7	28.1 ± 5.5	36.3 ± 2.6

TABLE VII (Continued)

	Black Pecan Aphid	Yellow Sugarcane Aphid	Greenbug (Biotype E)	Pea Aphid	Spotted Alfalfa Aphid	English Grain Aphid
12:0	0.3 ± 0.1	0.6	1.01 ± 0.1	0.6 ± 0.1	1.7 ± 0.1	1.0 ± 2.4
14:0	12.2 ± 2.4	20.0 ± 1.02	19.3 ± 0.3	9.9 ± 0.9	6.0 ± 0.4	31.6 ± 5.1
14:1	---	---	---	---	---	---
16:0	13.3 ± 1.7	0.8 ± 0.1	12.8 ± 0.4	0.4 ± 0.1	4.4	2.1 ± 0.7
16:1	0.1	---	--	---	0.1	T
18:0	2.3 ± 0.3	0.4 ± 0.1	1.5 ± 0.1	0.2	1.3 ± 0.1	0.4
18:1	1.2 ± 0.3	0.1	0.5	0.2	0.8	0.5 ± 0.2
18:2	0.8 ± 0.1	0.1	0.2	0.1	0.4	0.1 ± 0.1
γ18:3[1]	---	---	---	---	---	---
δ18:3[1]	---	---	---	---	---	---
β18:3[1]	---	---	---	---	---	T
α18:3[1]	0.1 ± 0.1	---	---	---	---	---
20:0	0.1	0.1	0.1	0.1 ± 0.1	0.1	T
Total	30.5 ± 4.3	22.0 ± 1.0	36.0 ± 1.8	11.6 ± 1.1	13.2 ± 0.4	35.9 ± 4.9

[1] Methyl esters of linolenic acid isomers confirmed by GC-MS. Note: n = 3, means ± SD, T = >0.05.

variance in the total μg of total lipid/mg sample. These ranged from 52.4 μg/mg for the birch aphid to 19.3 μg/mg for the pea aphid.

Neutral Lipid Fraction

In general, Table VII, containing μg of fatty acid per mg aphid, reflected its counterpart, Table IV, that contains percentages of fatty acids. The total μg of neutral lipid/mg also showed a large variation among the aphids. The giant bark aphid contained 9.2 μg/mg while the oleander aphid contained 36.3 μg/mg aphid.

Phospholipid Fraction

This table (Table VIII) containing μg of fatty acid per mg aphid, also reflected its counterpart, Table V, that contain percentages of fatty acids. The total amount of phospholipids/mg of an aphid were more consistent than either the total or neutral lipid fractions, as well as much less. The high was only 14.8 μg/mg and the low was 6.9 μg/mg.

% Composition of Linolenate Isomers in Other Insects

No linolenic acid isomers were found in the black scale or mealybugs (Table IX). The rest of the Hemiptera examined contained 100 % α-linolenic acid. The lesser grain borer, mealworm, alfalfa weevil and the spotted cucumber beetle contained only α-linolenic acid, while the southern masked chafer contained ca. 50% α and γ-linolenic acid. The two lady beetle species contained a mixture of

TABLE VIII

μg OF EACH FATTY ACID IN THE PHOSPHOLIPID FRACTION/mg APHID

	Giant Bark Aphid	Blue Alfalfa Aphid	Birch Aphid	Bird Cherry-Oat Aphid	Turnip Aphid	Russian Wheat Aphid	Oleander Aphid
12:0	0.1 ± 0.1	T	---	---	---	0.3 ± 0.1	---
14:0	1.1 ± 0.5	1.4 ± 1.3	0.4 ± 0.4	0.5 ± 0.2	1.7 ± 1.2	2.3 ± 1.4	0.3 ± 0.1
14:1	T	---	---	---	---	---	---
16:0	0.5 ± 0.1	0.3 ± 0.2	1.0 ± 0.4	0.4 ± 0.1	1.7 ± 1.2	0.9 ± 0.6	2.6 ± 0.6
16:1	0.4 ± 0.1	0.3 ± 0.1	0.2 ± 0.2	0.3 ± 0.1	0.5 ± 0.2	0.4 ± 0.4	0.4 ± 0.6
18:0	1.8 ± 0.1	1.2 ± 0.4	1.4 ± 0.3	1.1 ± 0.2	1.4 ± 0.3	1.6 ± 1.1	1.3
18:1	2.5 ± 0.3	1.8 ± 0.8	1.5 ± 0.3	1.3 ± 0.3	1.8 ± 0.8	1.9 ± 1.7	1.5 ± 0.2
18:2	4.4 ± 0.5	2.6 ± 1.1	2.8 ± 0.6	2.3 ± 0.5	2.8 ± 1.2	3.3 ± 3.0	2.7 ± 0.4
γ18:3[1]	---	---	---	---	---	---	---
δ18:3[1]	0.1	0.1	0.1	0.1	0.1	0.1 ± 0.1	0.1
ß18:3[1]	0.4	0.4 ± 0.2	0.3 ± 0.1	0.5 ± 0.1	0.5 ± 0.3	0.6 ± 0.5	0.3
α18:3[1]	---	---	---	---	---	---	---
20:0	0.5	0.4 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.7 ± 0.6	0.3
Total	11.4 ± 1.1	8.3 ± 4.1	7.9 ± 1.9	6.9 ± 1.2	10.8 ± 2.8	10.5 ± 1.5	9.7 ± 0.7

TABLE VIII (Continued)

	Black Pecan Aphid	Yellow Sugarcane Aphid	Greenbug (Biotype E)	Pea Aphid	Spotted Alfalfa Aphid	English Grain Aphid
12:0	T	T	0.1	0.1	---	0.2 ± 0.2
14:0	1.4 ± 0.2	2.0 ± 0.7	1.8 ± 0.5	1.1 ± 0.2	0.5 ± 0.1	5.6 ± 1.8
14:1	---	---	---	---	---	---
16:0	1.9 ± 0.1	0.2	1.4 ± 0.3	0.2	0.6 ± 0.1	0.7 ± 0.2
16:1	0.6 ± 0.1	0.2	0.4 ± 0.1	0.2	0.2	0.4 ± 0.1
18:0	2.0 ± 0.3	1.3 ± 0.1	1.3	1.0	1.6 ± 0.1	1.5 ± 0.1
18:1	2.7 ± 0.8	2.0 ± 0.5	2.0 ± 0.3	1.3 ± 0.2	1.8 ± 0.4	2.1 ± 0.5
18:2	3.5 ± 0.9	3.1 ± 0.7	2.7 ± 0.3	2.4 ± 0.4	3.6 ± 0.6	3.2 ± 0.8
γ18:3 [1]	---	---	---	---	---	---
δ18:3 [1]	0.1	0.1	0.1	T	0.1	0.1
ß18:3 [1]	0.2 ± 0.1	0.2	0.4	0.3 ± 0.1	0.3 ± 0.1	0.6 ± 0.2
α18:3 [1]	0.1	---	---	---	---	---
20:0	0.4 ± 0.6	0.5	0.6 ± 0.1	0.3	0.5 ± 0.1	0.4 ± 0.1
Total	12.8 ± 2.2	9.5 ± 1.0	10.5 ± 1.5	8.2 ± 2.8	9.3 ± 1.3	14.9 ± 2.2

[1] Methyl esters of linolenic acid confirmed by GC-MS. Note: n = 3, means ± SD, T = >0.05.

TABLE IX

% COMPOSITION OF LINOLENATE ISOMERS IN OTHER INSECTS[1] (TOTAL LIPID)

HEMIPTERA	γ18:3	δ18:3	β18:3	α18:3
Black Scale	---	---	---	---
Citrus Mealybug	---	---	---	---
Squash Bug (1st instar)	---	---	---	100.0
Squash Bug (unfed)	---	---	---	100.0
Small Milkweed Bug	---	---	---	100.0
Large Milkweed Bug	---	---	---	100.0
COLEOPTERA				
Lesser Grain Borer	---	---	---	100.0
Dark Mealworm (larvae)	---	---	---	100.0
Alfalfa Weevil	---	---	---	100.0
Spotted Cucumber Beetle (garden)	---	---	---	100.0
Spotted Cucumber Beetle (alfalfa)	---	---	---	100.0
Southern Mask Chafer	51.6 ± 12.1	T	---	48.4 ± 12.1
C7 Lady Beetle	---	23.0 ± 11.2	43.8 ± 6.2	33.2 ± 17.4
Spotted Lady Beetle	---	13.5 ± 1.7	12.2 ± 5.8	74.3 ± 7.4

TABLE IX (Continued)

LEPIDOPTERA	γ18:3	δ18:3	β18:3	α18:3
Milkweed Tiger Moth	---	---	---	100.0
Milkweed Tiger Moth (starved)	---	---	---	100.0
Fall Webworm (River Birch)	---	---	---	100.0
Fall Webworm (pecan)	---	---	---	100.0
Fall Webworm (starved)	---	---	---	100.0
Tobacco Hornworm	---	---	---	100.0
Bagworm	---	---	---	100.0
Greater Waxmoth	---	---	---	100.0
Alfalfa Leafworm	---	---	---	100.0
Yellow-Stripped Armyworm	---	---	---	100.0
Whitelined Sphinx	---	---	---	100.0
HYMENOPTERA				
Honey Bee (adult)	---	---	---	100.0
Honey Bee (pupae)	---	---	---	100.0
Honey Bee (larvae)	---	---	---	100.0
NEUROPTERA				
Green Lacewing	---	T	12.3 ± 3.9	87.7 ± 2.9

TABLE IX (Continued)

DIPTERA	γ18:3	δ18:3	β18:3	α18:3
Blow Fly (larvae)	84.3 ± 0.6	---	---	15.7 ± 0.6
Blow Fly (adults)	93.0 ± 1.3	---	---	7.0 ± 1.3
House Fly (males)	7.3 ± 1.0	---	---	92.7 ± 1.0
House Fly (female)	8.9 ± 4.4	---	---	91.1 ± 4.4
House Fly (larvae)	6.0 ± 2.3	---	---	94.0 ± 2.3
Horse Fly (atratus)	20.7 ± 10.0	---	---	79.3 ± 10.0
Horse Fly (abactor)	7.0 ± 9.8	---	---	90.3 ± 9.8
ORTHOPTERA				
Meadow Grasshopper	---	---	---	100.0
Field Cricket	---	---	---	100.0
DICTYOPTERA				
German Cockroach	---	---	---	100.0
American Cockroach (nymph)	T	---	---	100.0
American Cockroach (adult)	T	---	---	100.0

[1] The presence of linolenic acid isomers confirmed by GC-MS. Note: n = 3, means ± SD, 1T = >0.05.

α, β, and δ-linolenic acid. The α-linolenic acid occurred alone in Lepidoptera, Hymenoptera, Orthoptera, and Dictyoptera. Diptera contained the α and γ isomers in various proportions. The green lacewing contained 87.7% of the α isomer with 12.3% of the β isomer and a trace of the δ isomer.

Fatty Acid Composition of Total Lipids in Other Insects

% Composition of Fatty Acids

In the Order Hemiptera (Suborder Homoptera), black scale and citrus mealybugs has many similarities in their fatty acid makeup (Table X). In the Suborder Heteroptera, capric, lauric, and myristic acids were lacking and very little myristoleic acid was found in the insects studied. Fed and unfed 1st instar squash bugs had almost the same percentages of all fatty acids. Small and large milkweed bugs also contained similar percentages of fatty acids but showed the greatest differences in percentages of fatty acids compared to other Hemiptera with respect to proportions of palmitic, palmitoleic, stearic, oleic, and linoleic acids. The large milkweed bug contained a small percentage of behenic acid.

Coleoptera generally lacked capric, lauric, myristic, and behenic acids (Table X). The C7 lady beetle was alone in having more than 2.1% of myristic acid. The highest proportion of a fatty acid was oleic acid in all beetles analyzed. The two grain feeding insects examined, the lesser grain borer and the dark meal-worm, had very similar fatty acid profiles in terms of percentages.

The alfalfa weevil and the spotted cucumber beetles contained a high portion of α-linolenic acid while the other Coleoptera had very little. All beetles contained small percentages of arachidic acid.

In most Lepidoptera, the dominant fatty acids were palmitic, stearic, oleic, linoleic, and α-linolenic acids (Table X). However, in the tobacco hornworm and the greater wax moth, percentages of α-linolenic acid were very small, 1.8 and 0.5 %, respectfully. Some of the larvae of the milkweed tiger moth and the fall webworm were starved for 3 days to see how this affected α-linolenic acid content. Amounts of α-linolenic acid were about one-third lower in tiger moth larvae after starvation but were not changed much in the fall webworm. However, percentages of palmitic acid in both moths, decreased by more than one-half after starvation. This raised the percentages of stearic, oleic, and linoleic acids in these larvae. The tobacco hornworm had almost equal parts of oleic and linoleic acid while the wax moth's predominant fatty acid was linoleic followed by palmitic acid. In bagworm hemolymph, palmitic acid was almost twice as abundant as that in whole-body extracts. Percentages of other fatty acids were slightly lower than the whole body extract. The alfalfa leafworm, yellow-stripped armyworm and the white-lined sphinx all contained similar percentages of fatty acids.

In the Order Diptera, the highest percentages of fatty acids generally were palmitic, palmitoleic, and oleic acids (Table X). The horse fly (Tabanus abactor) exhibited high percentages of linoleic acid but low percentages of palmitoleic acid. Both blow fly larvae and adults contained fairly high percentages of arachidonic acid. The rest of the Diptera examined in this study, contained at least a trace arachidonic acid. These Diptera also contained a small amount of arachidic and eicosapentaenoic as well as α and γ-linolenic acids, with T. abactor having a high of 7.5% for α-linolenic acid.

TABLE X

% OF EACH FATTY ACID IN THE TOTAL LIPID EXTRACT OF OTHER INSECTS

HEMIPTERA

	Black Scale	Citrus Mealybug	Squash Bug (1st instar)	Squash Bug (unfed)	Small Milkweed Bug	Large Milkweed Bug
10:0	1.4 ± 0.4	1.3 ± 0.6	---	---	---	---
12:0	17.7 ± 1.0	33.7 ± 1.7	---	---	---	---
14:0	15.4 ± 1.4	12.5 ± 0.7	0.7 ± 0.1	0.7	0.1 ± 0.1	T
14:1	---	---	---	---	---	---
16:0	40.3 ± 2.0	2.2 ± 0.1	19.7 ± 0.2	19.8 ± 0.2	7.1 ± 0.9	6.3 ± 0.2
16:1	0.4	1.5	33.5 ± 1.2	36.0 ± 0.2	5.5 ± 1.6	T
18:0	5.0 ± 0.6	42.5 ± 1.5	10.9 ± 0.9	10.0 ± 0.1	7.9 ± 3.6	5.8 ± 0.9
18:1	6.1 ± 0.7	3.0 ± 0.1	34.2 ± 0.5	32.6 ± 0.5	19.4 ± 0.5	17.1 ± 1.7
18:2	12.2 ± 1.4	2.7 ± 0.1	0.3 ± 0.1	0.2	57.8 ± 7.8	69.8 ± 2.1
γ18:3[1]	---	---	---	---	---	---
δ18:3[1]	---	---	---	---	---	---
β18:3[1]	---	---	---	---	---	---
α18:3[1]	---	---	0.7	0.5	1.0 ± 1.2	0.1
20:0	1.0 ± 0.2	0.6	0.4 ± 0.1	0.3	0.3	0.5
20:4	---	---	---	---	---	---
20:5	---	---	---	---	---	---
22:0	T	---	T	T	---	0.4 ± 0.1

TABLE X (Continued)

COLEOPTERA

	Lesser Grain Borer	Dark Mealworm (larvae)	Alfalfa Weevil	Spotted Cucumber Beetle (garden)	Spotted Cucumber Beetle (alfalfa)	Southern Masked Chafer	C7 Lady Beetle	Spotted Lady Beetle
10:0	---	---	---	---	---	---	T	---
12:0	---	---	---	---	---	---	---	T
14:0	0.3	1.9 ± 0.5	0.4	0.3	T	---	14.2 ± 4.8	2.1 ± 0.8
14:1	---	---	---	---	---	---	---	---
16:0	25.9 ± 0.1	18.0 ± 1.3	9.4 ± 0.2	19.6 ± 0.9	19.6 ± 7.3	19.4 ± 2.4	3.6 ± 1.2	9.1 ± 0.5
16:1	0.3	1.5 ± 0.3	7.9 ± 1.5	0.8 ± 0.1	0.3 ± 0.2	2.4 ± 0.8	0.6 ± 0.2	0.9 ± 0.1
18:0	4.5 ± 0.2	3.8 ± 0.8	5.5 ± 0.8	7.3 ± 0.7	7.5 ± 0.8	4.2 ± 0.4	9.3 ± 0.3	17.8 ± 2.1
18:1	41.5 ± 0.1	52.8 ± 4.3	51.9 ± 1.1	39.8 ± 2.0	33.3 ± 7.6	57.0 ± 4.0	51.4 ± 3.2	54.7 ± 4.0
18:2	25.3 ± 0.2	21.8 ± 5.8	5.1 ± 0.5	13.7 ± 2.2	14.5 ± 1.0	12.7 ± 5.3	18.6 ± 2.1	12.3 ± 2.0
γ18:3[1]	---	---	---	---	---	0.7 ± 0.3	---	---
δ18:3[1]	---	---	---	---	---	T	0.3	0.3 ± 0.1
β18:3[1]	---	---	---	---	---	---	0.6 ± 0.2	0.2 ± 0.1
α18:3[1]	1.1 ± 0.1	0.3 ± 0.1	18.7 ± 0.9	17.0 ± 3.6	23.3 ± 6.9	0.7 ± 0.3	0.6 ± 0.5	1.5 ± 0.2
20:0	0.2	0.2 ± 0.1	0.6 ± 0.1	0.5	0.3 ± 0.3	0.2 ± 0.2	1.0 ± 0.1	1.3 ± 0.2
20:4	---	---	---	---	T	3.1 ± 1.8	---	---
20:5	---	---	---	---	---	---	---	---
22:0	---	---	---	---	---	---	---	---

TABLE X (Continued)

LEPIDOPTERA

	Bagworm	Bagworm (Hemolymph)	Greater Waxmoth	Alfalfa Leafworm	Yellow Stripped Armyworm	White-lined Sphinx
10:0	---	---	---	---	---	---
12:0	---	---	---	---	---	---
14:0	0.6 ± 0.1	---	0.2	T	T	T
14:1	---	---	---	---	---	---
16:0	17.3 ± 0.8	32.6 ± 12.1	37.4 ± 3.5	15.3 ± 1.6	12.0 ± 2.0	10.1 ± 2.2
16:1	0.9 ± 0.3	T	4.5 ± 0.2	T	T	T
18:0	8.1 ± 0.4	6.3 ± 1.2	2.3 ± 1.9	10.8 ± 1.6	9.0 ± 1.2	12.3 ± 2.8
18:1	22.6 ± 1.1	19.6 ± 3.6	46.2 ± 4.6	12.3 ± 1.3	13.9 ± 1.4	14.4 ± 0.2
18:2	15.3 ± 0.5	11.9 ± 2.2	4.6 ± 0.7	16.3 ± 2.0	17.3 ± 2.1	21.4 ± 2.8
γ18:3[1]	---	---	---	---	---	---
δ18:3[1]	---	---	---	---	---	---
ß18:3[1]	---	---	---	---	---	---
α18:3[1]	29.9 ± 1.7	26.6 ± 4.9	0.5 ± 0.1	43.5 ± 0.9	47.1 ± 1.3	39.8 ± 0.2
20:0	0.5 ± 0.1	---	4.2 ± 6.7	0.5 ± 0.5	T	0.8 ± 0.2
20:4	---	---	---	---	---	T
20:5	---	---	---	---	---	---
22:0	---	---	---	---	---	---

TABLE X (Continued)

LEPIDOPTERA

	Milkweed Tiger Moth	Milkweed Tiger Moth (Starved)	Fall Webworm (River Birch)	Fall Webworm (Pecan)	Fall Webworm (Starved)	Tobacco Hornworm
10:0	---	---	---	---	---	---
12:0	---	---	---	---	---	---
14:0	---	---	T	---	---	0.4
14:1	---	---	---	---	---	---
16:0	18.1 ± 5.0	6.8 ± 0.6	9.8 ± 0.2	15.6 ± 0.4	6.8 ± 0.6	17.8 ± 0.5
16:1	---	---	---		---	3.7 ± 0.3
18:0	8.1 ± 1.0	17.6 ± 0.8	13.4 ± 0.2	12.7 ± 0.2	15.4 ± 0.3	10.1 ± 1.0
18:1	9.2 ± 1.1	18.3 ± 3.1	8.5 ± 0.2	14.4 ± 0.2	17.0 ± 1.8	31.5 ± 1.2
18:2	11.7 ± 0.8	23.0 ± 0.5	16.9 ± 0.6	12.8 ± 0.1	19.9 ± 0.5	33.9 ± 0.6
γ18:3[1]	---	---	---	---	---	---
δ18:3[1]	---	---	---	---	---	---
β18:3[1]	---	---	---	---	---	---
α18:3[1]	51.7 ± 4.1	34.2 ± 2.1	49.3 ± 0.5	43.7 ± 0.1	42.9 ± 1.9	1.8 ± 0.1
20:0	---	---	0.8 ± 0.1	0.8	---	0.5
20:4	---	---	---	---	---	---
20:5	---	---	---	---	---	---
22:0	---	---	---	---	---	---

TABLE X (Continued)

DIPTERA

	Blow Fly (larvae)	Blow Fly (adults)	House Fly (males)	House Fly (females)	House Fly (larvae)	Horse Fly (atratus)	Horse Fly (abactor)
10:0	---	---	---	---	---	---	---
12:0	---	---	---	---	---	0.3 ± 0.2	---
14:0	7.4	1.0 ± 0.1	2.5 ± 0.5	2.2 ± 0.1	2.9 ± 0.1	0.7 ± 0.4	0.8 ± 0.1
14:1	---	---	T	0.6 ± 0.1	3.7	T	0.1 ± 0.1
16:0	18.5 ± 0.7	21.6 ± 0.5	26.7 ± 5.3	24.2 ± 0.5	28.7 ± 0.5	21.6 ± 6.3	19.3 ± 3.5
16:1	14.7 ± 2.3	15.7 ± 1.0	33.8 ± 5.9	30.5 ± 1.4	30.8 ± 1.4	16.1 ± 4.8	6.5 ± 1.6
18:0	6.1 ± 1.5	6.3 ± 1.0	7.1 ± 1.5	7.0 ± 0.5	10.7 ± 0.4	5.3 ± 2.0	5.9 ± 1.1
18:1	32.0 ± 0.7	28.7 ± 0.4	34.9 ± 8.8	29.3 ± 2.2	23.8 ± 1.1	34.1 ± 13.3	33.0 ± 5.1
18:2	9.0 ± 0.7	11.6 ± 0.2	5.0 ± 0.8	3.4 ± 0.4	2.1 ± 0.1	10.6 ± 5.7	20.8 ± 5.0
γ18:3[1]	0.7 ± 0.1	1.3 ± 0.1	1.1	T	T	0.8 ± 0.8	0.2 ± 0.1
δ18:3[1]	---	---	---	---	---	---	---
β18:3[1]	---	---	---	---	---	---	---
α18:3[1]	0.1	0.1	2.7 ± 0.2	0.8 ± 0.1	0.5	2.1 ± 1.4	7.5 ± 5.5
20:0	0.1	0.2	0.3	0.3	0.4	0.3 ± 0.1	3.3 ± 0.1
20:4	13.5 ± 0.8	10.7 ± 0.1	T	0.3 ± 0.1	T	3.7 ± 3.4	1.7 ± 0.7
20:5	1.5 ± 0.2	1.0 ± 0.1	0.6 ± 0.1	0.2	T	3.2 ± 2.6	1.0 ± 4.0
22:0	---	---	T	0.2 ± 0.1	---	---	---

TABLE X (Continued)

	ORTHOPTERA		DICTYOPTERA		
	Meadow Grasshopper	Field Cricket	German Cockroach	American Cockroach (nymph)	American Cockroach (adult)
10:0	---	---	---	---	---
12:0	---	---	T	---	---
14:0	0.8 ± 0.6	0.6 ± 0.2	0.5 ± 0.2	3.9 ± 0.3	0.4 ± 0.1
14:1	---	---	---	---	---
16:0	23.7 ± 5.8	15.4 ± 5.2	12.4 ± 6.5	14.7 ± 8.0	17.8 ± 2.5
16:1	3.8 ± 1.5	1.3 ± 0.8	2.2 ± 1.2	0.4 ± 0.1	1.0 ± 0.4
18:0	6.2 ± 0.4	3.1 ± 1.4	6.2 ± 0.9	9.3 ± 1.0	8.7 ± 1.8
18:1	35.1 ± 1.9	40.9 ± 8.5	50.1 ± 1.4	41.7 ± 4.7	44.3 ± 0.5
18:2	22.6 ± 5.0	28.1 ± 5.9	23.5 ± 5.5	27.7 ± 8.7	26.4 ± 1.9
γ18:3[1]	---	---	---	T	T
δ18:3[1]	---	---	---	---	---
β18:3[1]	---	---	---	---	---
α18:3[1]	7.9 ± 0.8	3.1 ± 1.8	0.4	0.4 ± 0.1	0.3
20:0	0.4 ± 0.1	0.5	T	0.4 ± 0.2	0.2 ± 0.1
20:4	---	---	3.6 ± 1.8	4.4 ± 2.9	1.3 ± 0.6
20:5	---	---	---	---	---
22:0	---	---	---	---	---

TABLE X (Continued)

	HYMENOPTERA			NEUROPTERA
	Honey Bee (adult)	Honey Bee (pupae)	Honey Bee (larvae)	Green Lacewing
10:0	---	---	---	---
12:0	---	---	T	---
14:0	---	2.5 ± 0.3	1.9 ± 0.1	7.4 ± 0.1
14:1	---	---	---	---
16:0	6.6 ± 1.9	32.5 ± 1.9	36.0 ± 0.5	27.4 ± 0.6
16:1	1.8 ± 0.3	0.5 ± 0.1	0.7 ± 0.1	0.6 ± 0.1
18:0	8.7 ± 0.2	13.8 ± 1.4	11.9 ± 0.8	3.6 ± 0.8
18:1	57.7 ± 3.7	47.9 ± 0.5	48.0 ± 1.3	53.2 ± 1.1
18:2	10.2 ± 1.8	1.1 ± 0.3	0.4 ± 1.9	13.7 ± 1.5
γ 18:3[1]	---	---	---	---
δ18:3[1]	---	---	---	T
ß18:3[1]	---	---	---	0.1 ± 0.1
α18:3[1]	14.6 ± 0.9	0.8 ± 0.4	0.8 ± 0.1	0.6 ± 0.1
20:0	---	0.5	0.3	0.3
20:4	---	---	---	---
20:5	---	---	---	---
22:0	---	---	---	---

[1] Methyl esters of linolenic acid confirmed by GC-MS. Note. n = 3, means ± SD, T = Trace = >0.05

The Orders Orthoptera and Dictyoptera had very similar fatty acid profiles with the exception that the cockroaches possessed some arachidonic acid and much smaller percentages of α-linolenic acid than did other species (Table X). All exhibited a high percentages of oleic acid.

Percentages of fatty acids in honey bee, (Order Hymenoptera), larvae and pupae were almost exactly alike but differed from the adult workers by containing some myristic acid, as well as considerable more palmitic acid and much less α-linolenic and linoleic acid (Table X). The larvae and pupae also contained a trace of arachidic acid.

In the green lacewing, (Order Neuroptera), oleic acid was the dominant fatty acid followed by palmitic, linoleic, and myristic acids. It also exhibited some palmitoleic, α, β, and a trace of δ-linolenic as well as arachidic acids (Table X).

μg of Fatty Acids/mg Insect

In the Suborder Homoptera, citrus mealybugs contained almost 3 times as much total fatty acid/mg insect as did black scale (Table XI). There were huge differences in the amounts of lauric and stearic acid in these two insects.

Fed and unfed squash bugs, (Suborder Heteroptera), had similar percentages of fatty acids but the unfed 1st instar nymphs contained ca. twice the amount of each fatty acid and almost two-thirds more total fatty acid/mg of insect as did nymphs that had fed (Table XI). Large milkweed bugs contained more of each fatty acid than were found in small milkweed bugs, but the large milkweed bug also contained almost twice as much linoleic acid and so was composed of almost one-third more total fatty acid/mg of insect.

The lesser grain borer and the dark mealworm (Order Coleoptera), had similar amounts of fatty acids per mg of insect except for oleic acid in which the mealworm contained 48.6 $\mu g/mg$ while the lesser grain borer had only 27.5 $\mu g/mg$ (Table XI). The alfalfa weevil contained the highest amounts of palmitoleic and oleic acids of all the Coleoptera in this study and the amounts of α-linolenic acid in these insects were the highest of all the insects studied. The spotted cucumber beetles collected from different habitats showed small differences at oleic and α-linolenic acids. They also exhibited less total fatty acid $\mu g/mg$ of insect than the other Coleoptera. The two lady beetle species had almost the same fatty acid profiles but when looking at the individual fatty acids, the spotted lady beetle possessed twice as much oleic acids and the C7 lady beetle exhibited more myristic acid. The southern masked chafer was the only Coleoptera that did not have any myristic and palmitoleic acid, but did have arachidonic acid.

With the exception of the greater wax moth, one of the characteristics of the Lepidoptera examined in this study was the low amount of total fatty acid/mg of insect Table XI). The greater wax moth had the most fatty acid per mg of tissue of all the insects studied. Another characteristic, excluding the wax moth and the tobacco hornworm, was α-linolenic acid being the most abundant fatty acid. The wax moth contained large amounts of oleic and palmitic acids compared to the rest of the Lepidoptera. The starved larvae of the milkweed tiger moth and the fall webworm showed the greatest decrease in amounts of palmitic and α-linolenic acids per mg of tissue.

All Diptera analyzed generally contained large amounts of oleic acid (Table

XI). The house fly females contained slightly greater amounts of fatty acids/mg of tissue than did the males, with larvae containing the greatest amounts of all. The female house fly had the most myristic acid of all the Diptera in the study. Blow fly adults showed a slight increase in total fatty acid/mg of tissue compared to the larvae, while both had the highest amount of arachidonic acid for all the insects analyzed.

Of the two Orthoptera examined in this study, the meadow grasshopper contained almost twice as much total fatty acid per mg of tissue as did the field cricket Table XI). The American cockroach had the least amount of total fatty acid per mg of tissue for all insects in this study (Table XI). Both the german and American cockroaches contained a trace of α-linolenic acid.

Honey bee (Hymenoptera) pupae and larvae, contained about the same amount of all fatty acids Table XI). The adults did not contain any myristic or arachidic acid as did both pupae and larvae. The adults contained greater amounts of α-linolenic acid than pupae and larvae but had very small amounts of total fatty acid/mg of insect. The green lacewing (Neuroptera), contained large amounts of oleic acid which was followed by palmitic acid (Table XI).

TABLE XI

μg OF EACH FATTY ACID IN THE TOTAL LIPID EXTRACT/mg INSECT

HEMIPTERA

	Black Scale	Citrus Mealybug	Squash Bug (1st instar)	Squash Bug (1st instar) unfed	Small Milkweed Bug	Large Milkweed Bug
10:0	0.2 ± 0.1	1.9 ± 0.9	---	---	---	---
12:0	9.0 ± 5.5	46.9 ± 1.0	---	---	---	---
14:0	7.9 ± 5.1	17.4 ± 1.8	0.4	0.8	0.1 ± 0.1	0.1 ± 0.1
14:1	---	---	---	---	---	---
16:0	20.2 ± 11.4	3.1 ± 0.3	10.0 ± 0.3	21.3 ± 0.9	5.3 ± 3.6	8.2 ± 1.1
16:1	0.2 ± 0.1	2.1 ± 0.1	16.9 ± 1.2	36.8 ± 2.2	0.9 ± 0.2	0.1
18:0	2.4 ± 1.2	59.2 ± 4.4	5.4 ± 0.1	10.7 ± 0.6	2.1 ± 0.2	8.6 ± 2.1
18:1	2.9 ± 1.5	4.2 ± 0.2	17.3 ± 0.4	35.1 ± 1.2	14.1 ± 10.1	21.7 ± 2.6
18:2	5.9 ± 3.0	3.7 ± 0.1	0.1	0.2	47.6 ± 39.9	89.8 ± 15.7
γ18:3[1]	---	---	---	---	---	---
δ18:3[1]	---	---	---	---	---	---
β18:3[1]	---	---	---	---	---	---
α18:3[1]	---	---	0.4	0.6	0.4 ± 0.1	0.1
20:0	0.5 ± 0.2	0.8	0.2	0.3	0.2 ± 0.2	0.6 ± 0.1
20:4	T	---	---	---	---	---
20:5	---	---	---	---	---	---
22:0	0.1 ± 0.1	---	---	---	0.1	0.5 ± 0.1
Total	49.9 ± 28.5	139.8 ± 6.9	50.6 ± 1.8	107.6 ± 5.0	76.4 ± 54.7	129.6 ± 20.5

TABLE XI (Continued)

COLEOPTERA

	Lesser Grain Borer	Dark Mealworm (larvae)	Alfalfa Weevil	Spotted Cucumber Beetle (garden)	Spotted Cucumber Beetle (alfalfa)	Southern Masked Chafer	C7 Lady Beetle	Spotted Lady Beetle
10:0	---	---	---	---	---	---	---	---
12:0	---	---	---	---	---	---	---	T
14:0	0.2	1.6 ± 0.1	0.6 ± 0.1	0.1	T	---	4.4 ± 2.7	1.1 ± 0.2
14:1	---	---	---	---	---	---	---	---
16:0	17.2 ± 0.3	16.6 ± 10.1	13.0 ± 2.5	4.6 ± 0.3	5.7 ± 2.6	5.9 ± 3.2	1.2 ± 0.7	5.0 ± 0.8
16:1	0.2	1.4 ± 0.7	11.1 ± 4.1	0.2	T	---	0.2 ± 0.1	0.5 ± 0.1
18:0	3.0 ± 0.1	3.7 ± 2.7	7.4 ± 0.9	1.7 ± 0.2	2.1 ± 0.3	0.8 ± 0.6	2.5 ± 1.1	9.4 ± 1.9
18:1	27.5 ± 0.4	48.6 ± 28.8	71.8 ± 16.4	9.5 ± 0.8	8.0 ± 3.1	17.1 ± 8.3	14.3 ± 5.5	30.3 ± 9.0
18:2	17.2 ± 0.1	18.0 ± 7.9	6.9 ± 0.7	3.2 ± 0.4	4.1 ± 0.8	3.3 ± 0.8	5.1 ± 1.9	6.6 ± 0.6
γ18:3[1]	---	---	---	---	---	0.1 ± 0.1	---	---
δ18:3[1]	---	---	---	---	---	---	0.1	0.1
β18:3[1]	---	---	---	---	---	---	0.2 ± 0.1	0.1
α18:3[1]	0.7	0.3 ± 0.1	25.6 ± 4.1	4.2 ± 0.9	6.4 ± 0.4	0.2	0.1 ± 0.1	0.8 ± 0.3
20:0	0.1	0.2 ± 0.2	0.6	0.1	T	T	0.3 ± 0.1	0.7 ± 0.2
20:4	---	---	---	---	---	0.8 ± 0.2	---	---
20:5	---	---	---	---	T	---	---	---
22:0	---	---	---	---	---	---	---	---
Total	66.7 ± 1.0	90.5 ± 50.6	137.4 ± 28.6	23.8 ± 0.9	30.5 ± 7.8	29.6 ± 12.3	28.5 ± 12.1	54.8 ± 12.2

TABLE XI (Continued)
LEPIDOPTERA

	Bagworm	Greater Waxmoth	Alfalfa Leafworm	Yellow-Stripped Armyworm	Milkweed Tiger Moth	Milkweed Tiger Moth (starved)
10:0	---	---	---	---	---	---
12:0	---	---	---	---	---	---
14:0	0.1	0.4 ± 0.1	T	T	---	---
14:1	---	---	---	---	---	---
16:0	2.8 ± 0.1	69.1 ± 4.9	1.9 ± 0.8	0.8 ± 0.5	1.8 ± 0.4	0.3 ± 0.1
16:1	0.1	8.2 ± 0.1	T	---	---	---
18:0	1.3 ± 0.1	4.5 ± 3.7	1.3 ± 0.5	0.5 ± 0.1	1.0 ± 0.1	0.8 ± 0.2
18:1	3.8 ± 0.4	85.3 ± 4.6	1.5 ± 0.5	0.9 ± 0.5	1.2 ± 0.3	0.8 ± 0.2
18:2	2.4 ± 0.3	8.6 ± 1.1	1.9 ± 0.5	1.0 ± 0.3	1.5 ± 0.2	1.0 ± 0.2
γ18:3[1]	---	---	---	---	---	---
δ18:3[1]	---	---	---	---	---	---
β18:3[1]	---	---	---	---	---	---
α18:3[1]	4.5 ± 0.5	1.0 ± 0.1	5.2 ± 1.6	2.8 ± 1.1	6.7 ± 0.5	1.5 ± 0.3
20:0	0.1	---	---	---	--	---
20:4	---	---	---	---	---	---
20:5	---	---	---	---	---	---
22:0	---	---	---	---	---	---
Total	15.1 ± 0.5	177.0 ± 6.0	11.9 ± 3.9	6.0 ± 2.6	12.8 ± 1.6	4.4 ± 0.8

TABLE XI (Continued)

DIPTERA

	Blow Fly (larvae)	Blow Fly (adults)	House Fly (males)	House Fly (females)	House Fly (larvae)	Horse Fly (abactor)	Horse Fly (atratus)
10:0	---	---	---	---	---	---	---
12:0	---	---	---	T	---	---	T
14:0	0.2	0.4	0.5 ± 0.1	7.7 ± 0.1	1.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
14:1	---	---	---	---	---	---	---
16:0	5.8 ± 0.4	8.3 ± 0.6	5.8 ± 1.0	8.4 ± 0.7	12.7 ± 1.4	7.6 ± 2.4	4.6 ± 2.7
16:1	5.0 ± 0.2	6.0 ± 0.5	7.3 ± 1.0	10.6 ± 1.1	13.9 ± 0.9	2.5 ± 0.4	3.8 ± 2.5
18:0	1.6 ± 0.1	2.4 ± 0.4	1.5 ± 0.3	2.4 ± 0.3	4.9 ± 0.3	2.3 ± 0.3	1.0 ± 0.3
18:1	9.9 ± 0.8	11.0 ± 0.6	7.6 ± 1.6	10.1 ± 0.9	10.8 ± 1.4	12.6 ± 0.6	8.0 ± 6.1
18:2	2.9 ± 0.3	4.5 ± 0.3	1.1 ± 0.2	1.2 ± 0.1	0.9 ± 0.1	8.1 ± 2.3	1.9 ± 0.9
γ18:3[1]	0.2	0.5 ± 0.5	T	T	T	0.1	0.1 ± 0.1
δ18:3[1]	---	---	---	---	---	---	---
ß18:3[1]	---	---	---	---	---	---	---
α18:3[1]	T	T	0.3 ± 0.1	0.3	0.2	3.0 ± 2.2	0.4 ± 0.2
20:0	T	0.1	0.1	0.1	0.2	0.1	0.1
20:4	3.3 ± 0.4	4.1 ± 0.2	T	0.1	---	0.6 ± 0.3	0.6 ± 0.3
20:5	0.4 ± 0.1	0.4	T	T	T	0.4 ± 0.2	0.6 ± 0.2
22:0	---	---	---	T	T	---	---
Total	30.1 ± 3.3	37.6 ± 2.2	24.3 ± 4.0	34.0 ± 2.5	45.0 ± 4.2	38.8 ± 6.2	21.3 ± 10.3

TABLE XI (Continued)

LEPIDOPTERA

	Fall Webworm (River Birch)	Fall Webworm (Pecan)	Fall Webworm (Pecan) (starved)	Tobacco Hornworm	White-lined Sphinx
10:0	---	---	---	---	---
12:0	---	---	---	---	---
14:0	T	---	---	0.1	---
14:1	---	---	---	---	---
16:0	0.5 ± 0.1	2.5 ± 0.1	0.1	2.7 ± 0.4	0.6 ± 0.3
16:1	---	0.1	---	0.6	---
18:0	0.7 ± 0.1	2.0 ± 0.1	0.4 ± 0.1	1.6 ± 0.3	0.7 ± 0.4
18:1	0.4 ± 0.1	2.2 ± 0.2	0.4 ± 0.1	4.8 ± 0.6	0.8 ± 0.5
18:2	0.9 ± 0.2	2.0 ± 0.1	0.5 ± 0.1	5.2 ± 0.6	1.2 ± 0.6
γ18:3[1]	---	---	---	---	---
δ18:3[1]	---	---	---	---	---
β18:3[1]	---	---	---	---	---
α18:3[1]	2.4 ± 0.4	7.0 ± 0.5	1.1 ± 0.1	0.3	2.3 ± 1.3
20:0	T	0.1	---	---	---
20:4	---	---	---	---	---
20:5	T	---	---	---	---
22:0	---	---	---	---	---
Total	5.8 ± 0.9	16.9 ± 2.2	2.5 ± 0.3	15.7 ± 1.8	5.6 ± 3.0

TABLE XI (Continued)

	ORTHOPTERA		DICTYOPTERA		
	Meadow Grasshopper	Field Cricket	German Cockroach	American Cockroach (nymph)	American Cockroach (adult)
10:0	---	---	---	---	---
12:0	T	---	---	---	---
14:0	0.5 ± 0.2	0.1	0.1 ± 0.2	T	T
14:1	---	---	---	---	---
16:0	7.8 ± 4.1	2.8 ± 1.4	3.6 ± 4.5	2.7 ± 4.0	1.3 ± 0.7
16:1	1.3 ± 0.6	2.2 ± 0.1	0.6 ± 0.8	T	0.7 ± 0.8
18:0	2.1 ± 0.7	1.5 ± 0.5	1.3 ± 1.1	1.1 ± 1.4	0.6 ± 0.2
18:1	11.9 ± 3.4	7.1 ± 0.5	10.9 ± 9.3	5.7 ± 7.5	3.0 ± 1.2
18:2	7.4 ± 0.8	5.2 ± 2.3	4.5 ± 2.8	2.6 ± 2.4	1.7 ± 0.6
γ18:3[1]	---	---	---	---	---
δ18:3[1]	---	---	---	---	---
β18:3[1]	---	---	---	---	---
α18:3[1]	2.7 ± 1.0	0.7 ± 0.4	0.1 ± 0.1	T	T
20:0	0.1	0.1	---	T	T
20:4	---	---	0.6 ± 0.2	0.1 ± 0.1	0.1
20:5	---	---	---	---	---
22:0	---	---	---	---	---
Total	33.5 ± 10.7	17.9 ± 4.0	21.8 ± 19.0	12.5 ± 15.4	6.7 ± 2.8

TABLE XI (Continued)

	HYMENOPTERA			NEUROPTERA
	Honey Bee (adult)	Honey Bee (pupae)	Honey Bee (larvae)	Green Lacewing
10:0	---	---	---	---
12:0	---	---	T	---
14:0	---	0.8 ± 0.2	0.6 ± 0.1	0.6 ± 0.1
14:1	---	---	---	---
16:0	0.7 ± 0.2	10.3 ± 1.8	10.8 ± 1.7	16.7 ± 1.8
16:1	0.2 ± 0.1	0.1	0.2 ± 0.1	0.4 ± 0.1
18:0	1.0 ± 0.1	4.4 ± 1.3	3.6 ± 0.7	2.5 ± 0.9
18:1	6.5 ± 1.0	15.2 ± 3.2	14.3 ± 1.7	32.5 ± 1.7
18:2	1.1 ± 0.2	0.4 ± 0.2	0.1 ± 0.1	9.4 ± 0.2
γ18:3[1]	---	---	---	---
δ18:3[1]	---	---	---	T
β18:3[1]	---	---	---	T
α18:3[1]	1.6 ± 0.1	0.2	0.2 ± 0.1	0.4
20:0	---	0.2	0.1	0.3
20:4	---	---	---	---
20:5	---	---	---	---
22:0	---	---	---	---
Total	11.1 ± 1.0	31.6 ± 6.6	29.7 ± 4.3	62.2 ± 3.4

[1] Methyl esters of linolenic acid confirmed by GC-MS. Note: n = 3, means ± SD, T = >0.05.

CHAPTER V

DISCUSSION

In a very broad view, the major biochemical pathways and mechanisms are very similar in insects and vertebrates. But, exceptions in insects have been found quite often in the past few years. Insects have not been studied as intensely as vertebrates and many researchers, previously believing that all insects behaved biochemicaly as vertebates, have had to make adjustments to all the new information. Insects make ideal models for research in many different areas. They are cheap and easy to rear. They grow fast and can be tested in quantity, thus eliminating individual variation.

A thorough literature search failed to find any references to other isomers of linolenic acid besides α and γ. Beta and delta isomers elute close to the α-linolenic acid, so previous researchers probably assumed they were the α isomer. Unless these two isomers are found in plants, it is probable that they are biosynthesized by aphids. The PL samples of the aphids and the TL samples of other insects were sent to Dr. Ralph Howard in Manhattan, KS, for gas chromatography-mass spectrometry confirmation of the isomers of linolenic acid. The presence of β and δ isomers in all aphids were confirmed as were those of the two lady beetle species and the green lacewings. The other insects contained only α and/or γ-linolenic acid. Black scales (Fig. 22), spotted cucumber beetles

(Fig. 29 & 30), house flies (Fig. 49, 50, & 51), horse fly (Fig. 52), and the American cockroaches (Fig. 57 & 58) appeared to have the δ isomer of linolenic acid but GC-MS showed this peak to be nonadecanoic acid (19:0) which eluted in the same area as the δ isomer on the GC. The large milkweed bugs (Fig. 26) and the field crickets (Fig. 55) appeared to have both β and δ isomers of linolenic acid while most of the other insects appeared to have the β isomer which proved to be 9-nonadecenoic acid (19:1). 9-Nonadecenoic acid elutes in the same area as the β-linolenic acid on the GC. It seems that with these isomers, one does not occur without the other one and since they have not been reported in plants, it is reasonable to assume that the isomers are synthesized by aphids. Lady beetles and green lacewings probably obtain the isomers from aphids in their diet. Each C7 lady beetle was collected in sorghum feeding on aphids. It would be interesting to analyze lady beetles reared on a different diet.

At the present time, it is not known how or why aphids make an isomer of linolenic acid with double bonds in the 9,12, and 17 positions. The positions of the double bonds of δ-linolenic acid have not yet been determined. In all aphids analyzed, the β isomer of linolenic acid has always been accompanied by the δ isomer. All the isomers of linolenic acid occurred predonimantly in the PL fraction, so they are probably involved in membrane structures and/or used as precursors for prostaglandins and their derivatives.

In general, aphids exhibited 9 basic fatty acids in the TL with few exceptions. Those 9 are myristic, palmitic, palmitoleic, stearic, oleic, linoleic, β and δ-linolenic, and arachidic acids. The most prominent fatty acids were myristic and/or palmitic followed by linoleic, oleic, and stearic acid. Within aphids as a group, there was great variation in the amount of individual fatty acids as well as total fatty acid per mg tissue. This was also true for the percentages of fatty acids. There seems to be three different groups among the aphids analyzed. One group displayed high percentages and amounts of myristic acid and low palmitic acid (blue alfalfa, Russian wheat, yellow sugarcane, pea, English grain, and giant bark aphids). A second group contained the reverse of the first (birch and oleander aphids) while a third group contained similar percentages and amounts of those two fatty acids with myristic acid being higher (greenbugs, chrysanthemum, bird cherry-oat, turnip, and spotted alfalfa aphids). The black pecan aphids are similar to the third group except that palmitic acid was higher as well as being the only aphid to possess α-linolenic acid. The three aphids containing palmitic acid as the dominant fatty acid (birch, oleander, and black pecan aphids) also use trees or shrubs as their host during part of their life cycle. This may account for the difference in their fatty acid content.

The results of the present study generally agree with the lipid reviews by Fast (1964 and 1970) and Gilbert (1967) except that all reviews reported an absence of linolenic acid and did not look for fatty acids beyond the 18 carbon length. The data from the present study on the oleander aphid was totally different from the study by Strong (1963b). That study used only apterae aphids from the oleander whereas this study used a mixture of nymphs and adults from the climbing milkweed. The dominant fatty acid in this study was palmitic acid at 66.3 % but myristic acid was dominant in the Strong study. From the present study, all other fatty acids were higher in addition to having β and δ-linolenic acid and arachidic acid present. This could be due to the difference in the host and life stages of the aphids used in the two studies. It has been shown that the host does effect the fatty acid composition of the spotted alfalfa aphid (Bergman, et al., in press). Two other aphids used in both studies were the bird cherry-oat and the spotted alfalfa aphids. The data for these also differed condiderably. Small amounts of linolenic acid were found in Strong's study but no indication as to which isomer was present. The present study found only a small percent of arachidic acid (2.5%), whereas Strong reported 10.1%.

A study on the effect of age and the symbiont population on the biosynthesis of lipid in the pea aphid (de Renobales, et al., 1990), reported that in the phospholipid fraction, stearic, oleic, and linoleic acids decreased with age but linolenic acid remained relatively constant. The data of Stephen and Gilbert (1970) suggested that individual variation in the composition is less for phospholipids than neutral lipids. This is in agreement with the data from the present study. The data reported by Gilbert (1967) generally agrees with the present study except that linolenic acid was reported absent.

Thompson (1973) reported that Dictyoptera lacked polyunsaturated fatty acids which differs with the findings of this study and also those of Blomquist, et al. (1983), Stanley-Samuelson and Pipa (1984) and Jurenka, et al. (1987).

Most of the previous studies of fatty acids in insects have been on a percentile basis (Thompson, 1973). A different perspective can be received when comparing amounts of fatty acids in insects. Two insects may have similar fatty acid percentages but the amounts per mg of tissue for each may be different.

The pea aphid and the blue alfalfa aphid are from the same genus and their percentages of fatty acids are much the same. However, blue alfalfa aphids have about one-third more total fatty acid, on a weight basis, than the pea aphids in their TL.

The chromatograms for all insects other than aphids may be found in the appendix (p. 97). The unfed 1st instar squash bug nymphs evidently contain large stores of most fatty acids from the eggs because the fed nymphs contains lower amounts fatty acids. The large milkweed bugs were reared on sunflower seeds. It would be interesting to see if those collected from the field have the same percent composition and amount of fatty acids per mg of tissue as those from the colony. The large milkweed bug contained very little linolenic acid and almost twice as much linoleic acid as the small milkweed bug which was collected in the field. The large milkweed bug contained 0.4% of 22:0, which was not reported by Gilbert (1967).

The coleopterans also exhibited great variation in their fatty acid content. The alfalfa weevil and the spotted cucumber beetle, as foliage feeders, displayed higher percentages of α-linolenic acid compared to the rest of the beetles. The southern masker chafer may have a Δ^6 and a Δ^{15} desaturase since they contain both α and γ-linolenic acid.

With the exceptions of the wax moth and the tobacco hornworm, the dominant fatty acid in Lepidoptera larvae was α-linolenic acid. This was expected since they are voracious foliage feeders. The hornworms were colony reared on artificial diet so this is another insect whose fatty acid content may be different when feeding on their natrual host. Except for the wax moth, the Lepidoptera contained the lowest amount of total fatty acid per mg of tissue. The data from the present study generally agrees with that found in several reviews (Fast, 1964 and 1970; Gilbert, 1967). Fall webworms collected from the pecan appear to do better than those collected from the river birch and contain more than double the amount of fatty acid. The present study found myristic and palmitoleic acids absent, but a review by Gilbert (1967) reported 1% for both acids. The starved webworms and milkweed tiger moth larvae seemed to have equally reduced amounts of all fatty acids.

The Diptera were probably the most interesting because their chromatograms contained many unknown peaks. All the Diptera contained the longer chained polyunsaturated fatty acids as well as both α and γ-linolenic acid. The percentages are deceiving in the house flies. The house fly larvae have the highest amount of fatty acid per mg of tissue. These fatty acids could be used during the pupal stage. The females have more fatty acids than the males. Female house flies may require these fatty acids for vittellogensis and egg laying. Reviews by Fast (1964 and 1970) reported linolenic acid (not quantified and 1.4%) but does not indicated which isomer was present. The present study found small percentages of 20:0, 20:4, 20:5, and 22:0, whereas the above reviews did not look for fatty acids with carbon chain lengths beyond 18 carbon. The blow flies contained the highest amounts of arachidonic acid compared to all other insects in this study.

Many of the insect species were collected on different dates, including some of the colony reared insects. Some of these groups have a higher standard deviation for some of their fatty acids. Very few of the specimens were sexed and the larvae collected in the field were not all the same instar. Factors such as these may have contributed to the variation observed.

SUMMARY AND CONCLUSIONS

Aphids and other insects were collected in the field and from various established colonies. Whole body lipid extracts were split into TL, NL, and PL fractions and made into FAMEs. Only TL fractions were analyzed from insects other than aphids. These fractions were analyzed to determine the percent composition and the amount of each fatty acid per mg of insect tissue. The occurrence and distribution of all isomers of linolenic acid were determined and recorded in TL, NL, and PL fractions of samples in aphids but only the TL fraction of the other insects. The fatty acid composition by percentage and amount per mg of insect tissue was recorded for all insects. This was important because there were no such records in literature.

The PL fraction of all aphids contained most of the linolenic acid. The NL fraction contained most of the shorter chain fatty acids which was primarily myristic and palmitic acids. The PL fraction contained most of the stearic, oleic, linoleic, linolenic, arachidic, and arachidonic acids.

It was found that β and δ-linolenic acid appears never to occur one without the other. So far, the β isomer has always been dominant. These two isomers were only found in aphids and insects that were predators upon aphids, lady beetles and green lacewings. Except for the black pecan aphid, α-linolenic acid was absent in aphids and γ-linolenic acid did not appear in any aphid. All

Lepidoptera, Hymenoptera, Othoptera, Dictyoptera, Heteroptera, and most Coleoptera contained α-linolenic acid. The Diptera and the southern masked chafer contained both α and γ-linolenic acid.

It is important to learn as much as possible about insects so as to be better able to control them. A way to control aphids may be found by learning how and why aphids synthesize β and δ-linolenic acid. Each species of insect seems to have their own fatty acid 'print' but each needs a greater number analyzed. Where possible, age, sex, host or diet should be determined and compared.

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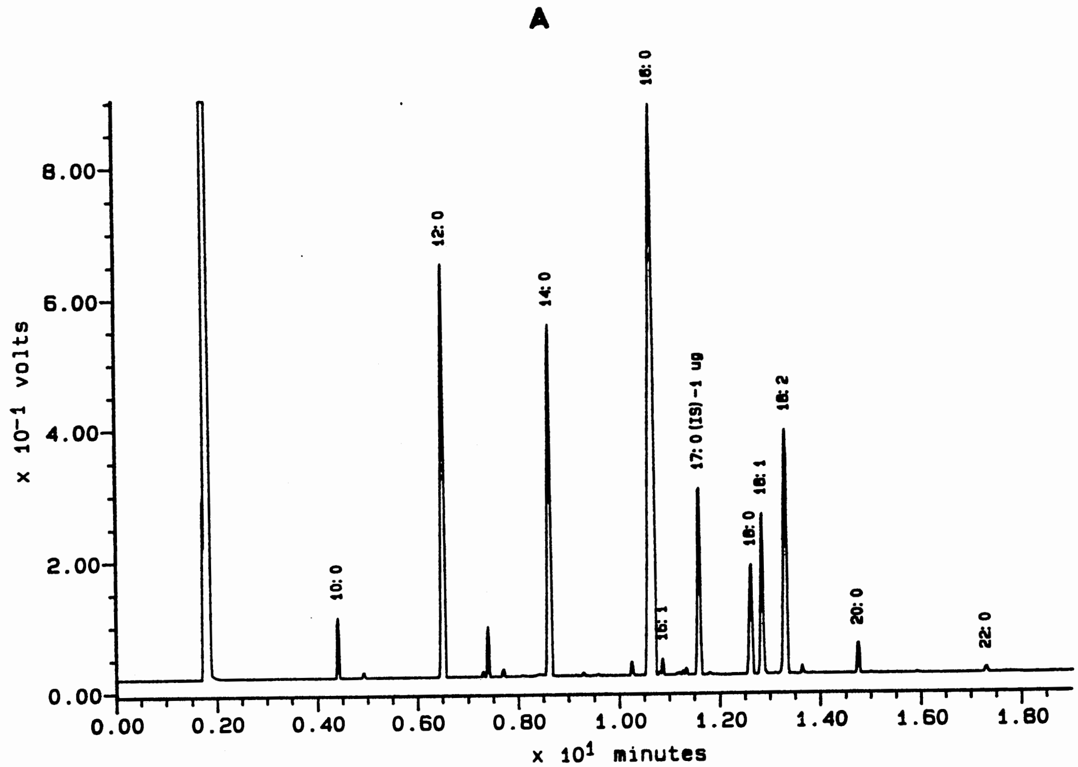
APPENDIX

GC TRACES OF THE TOTAL LIPID FAMEs AND THE 18:3 AREAS FOR INSECTS OTHER THAN APHIDS



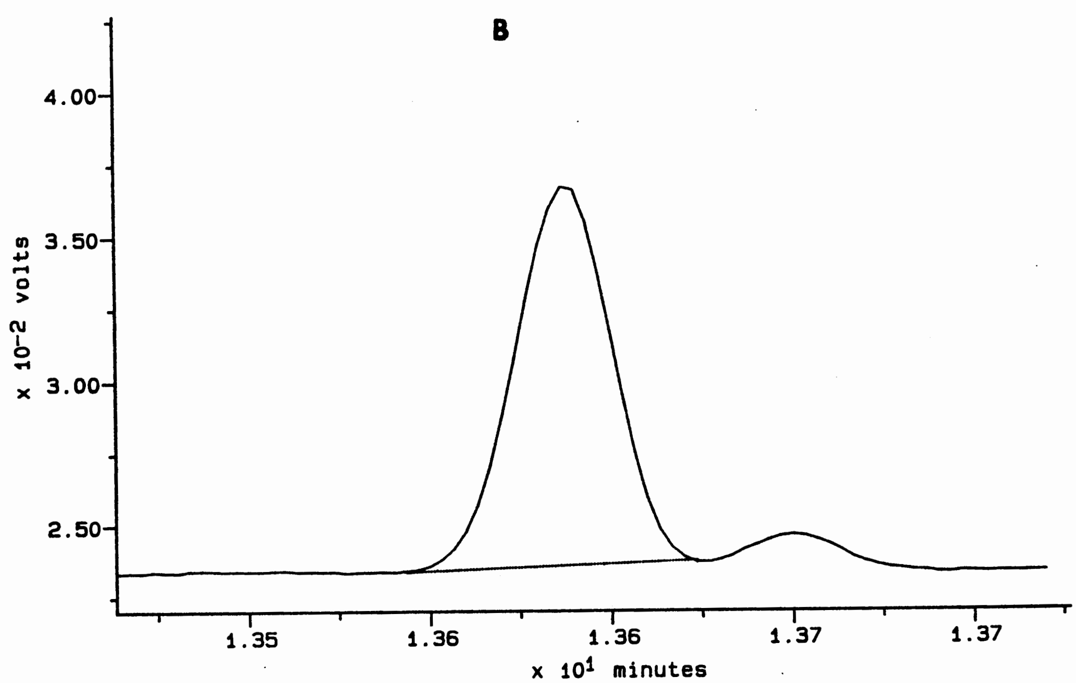


Figure 22. GC Trace of the Total Lipid FAME of the Black Scale (Saissetia oleae) (A) and the 18:3 Area of that Trace (B)

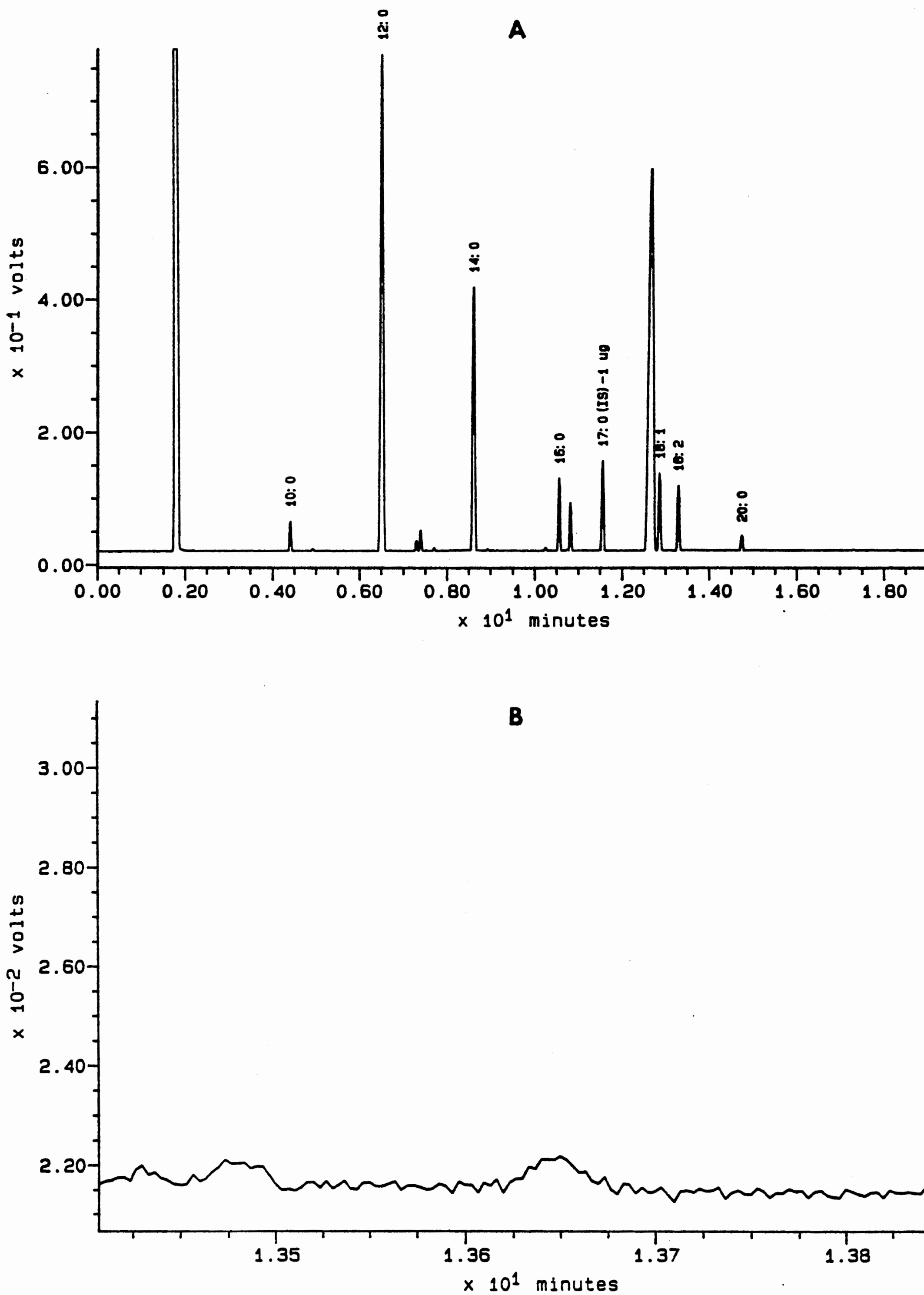
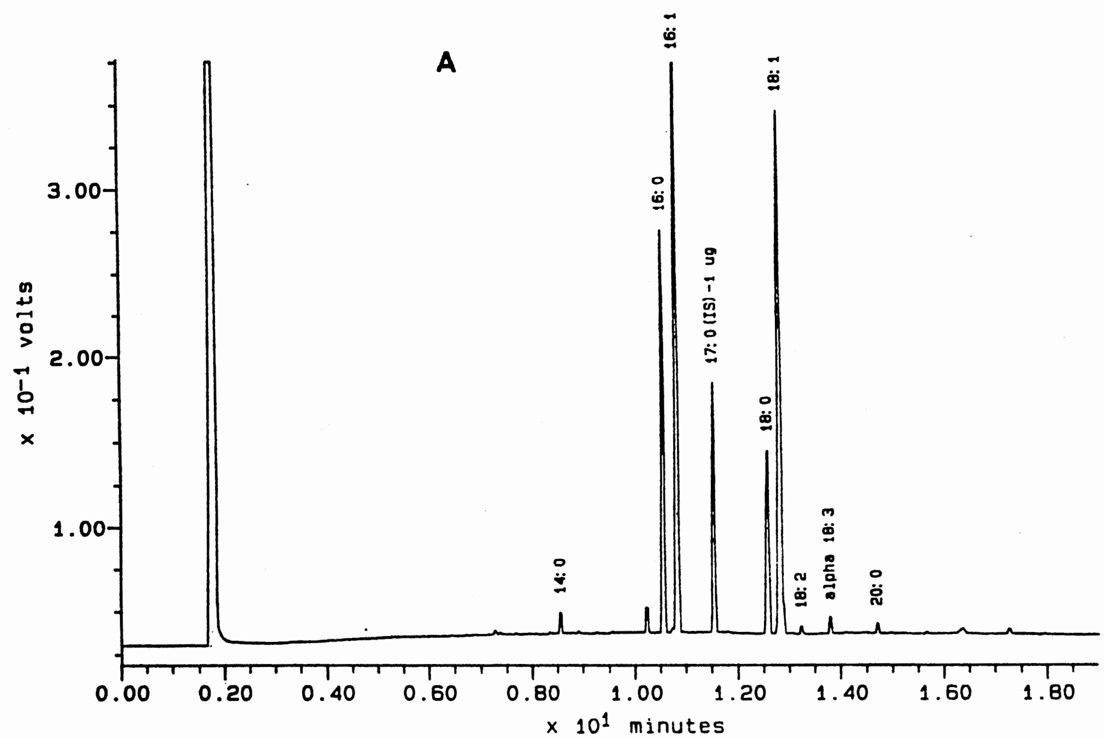


Figure 23. GC Trace of the Total Lipid FAME of the Citrus Mealybug (Planococcus citri) (A) and the 18:3 Area of that Trace (B)



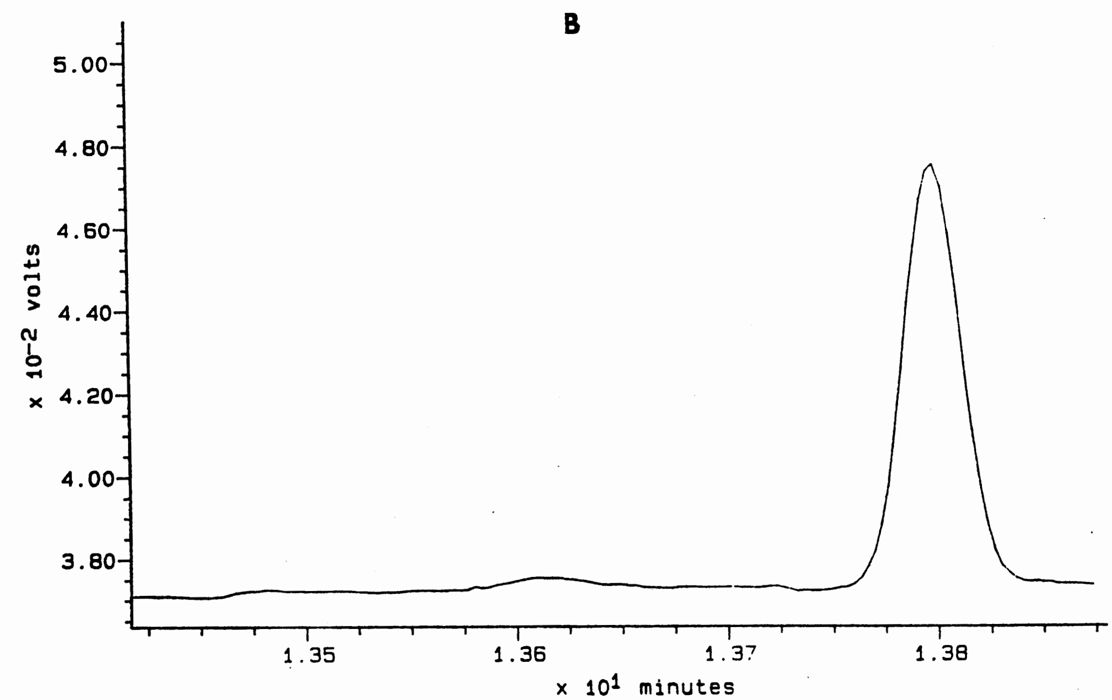


Figure 24. GC Trace of the Total Lipid FAME of the Squash Bug (Anasa Tristis) (A) and the 18:3 Area of that Trace (B)

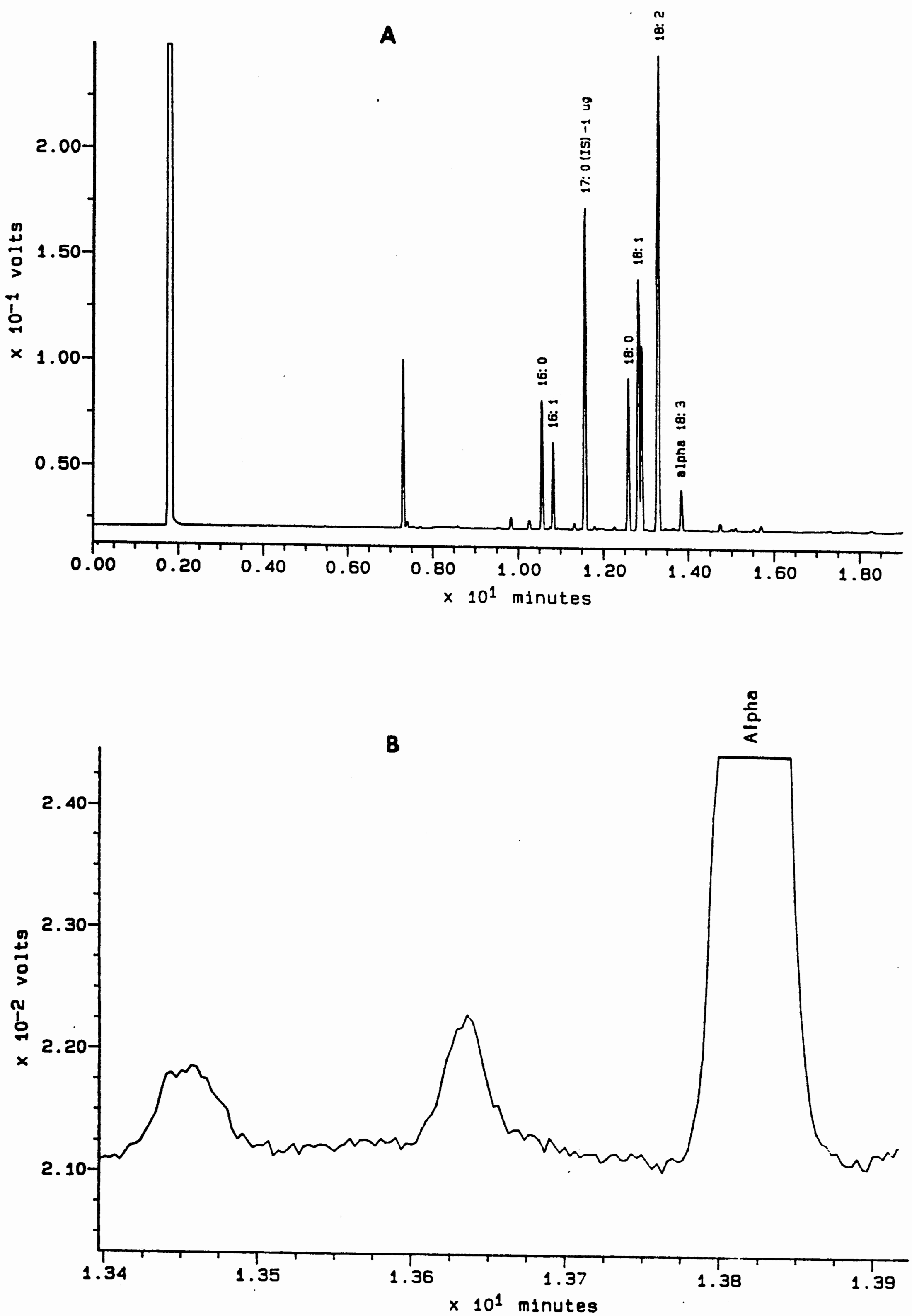


Figure 25. GC Trace of the Total Lipid FAME of the Small Milkweed Bug (<u>Lygaeus</u> <u>kalmii</u>) (A) and the 18:3 Area of that Trace (B)

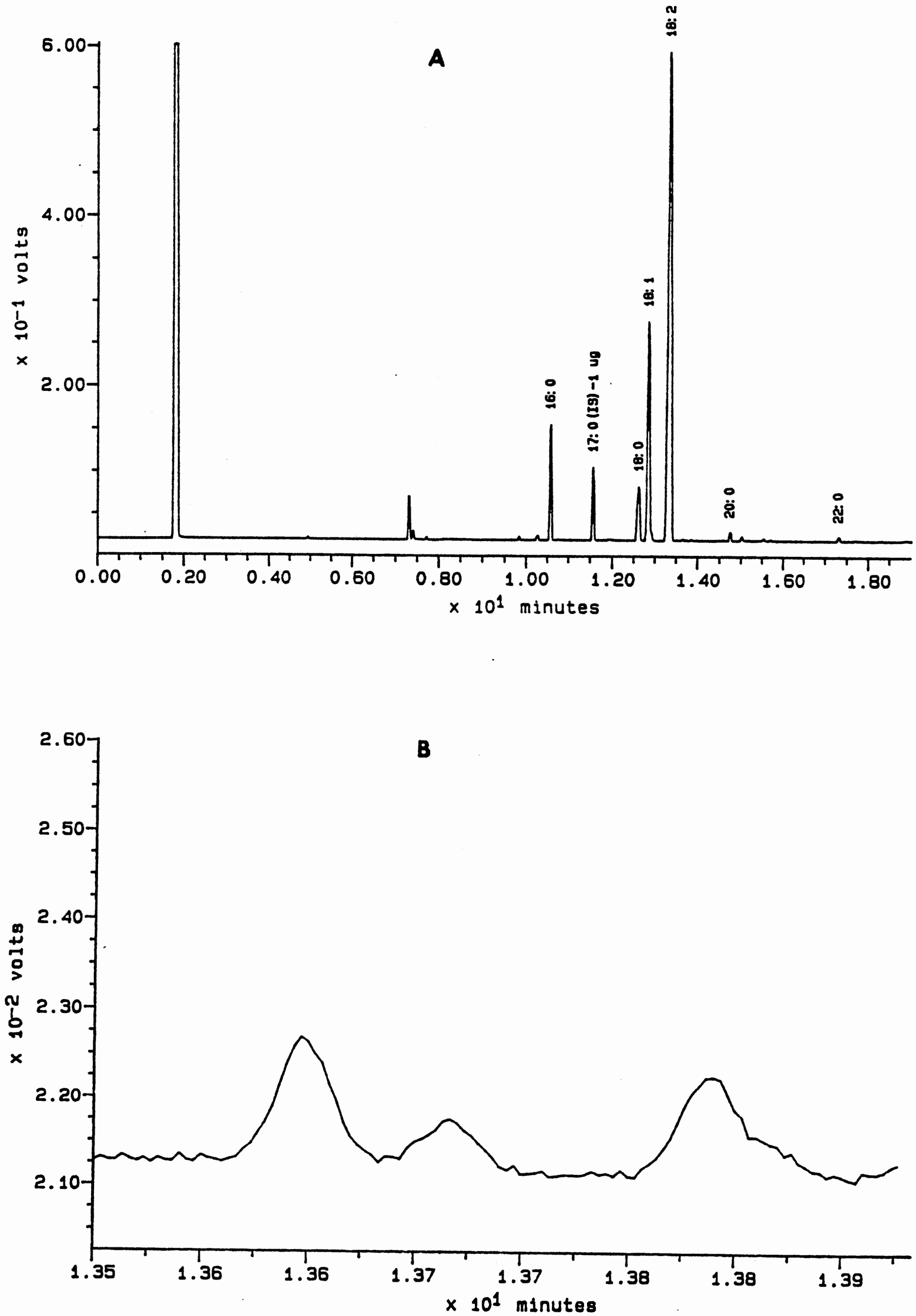


Figure 26. GC Trace of the Total Lipid FAME of the Large Milkweed Bug (*Oncopeltus fasciatus*) (A) and the 18:3 Area of that Trace (B)

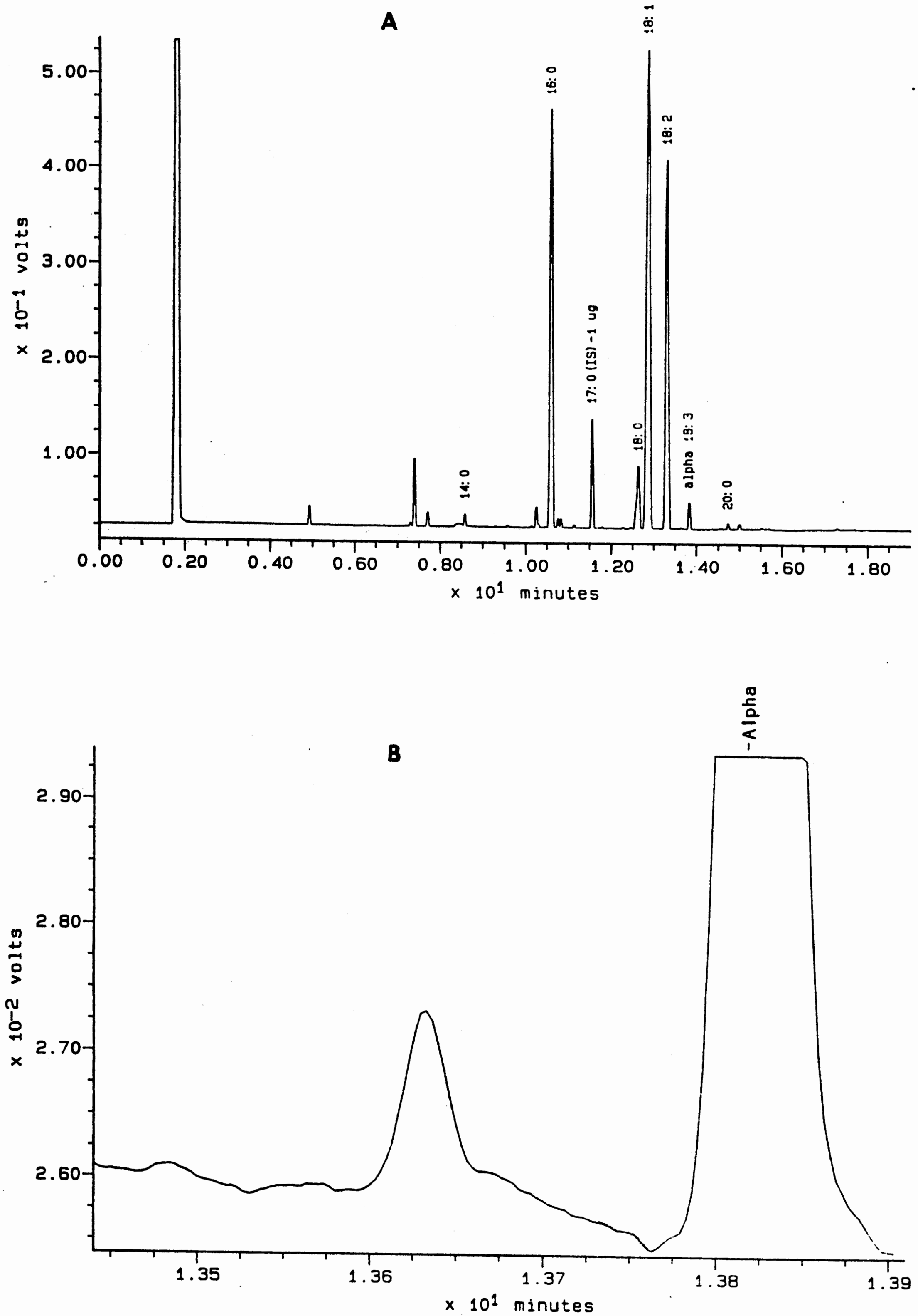


Figure 27. GC Trace of the Total Lipid FAME of the Lesser Grain Borer (Rhizopertha dominica) (A) and the 18:3 Area of that Trace (B)

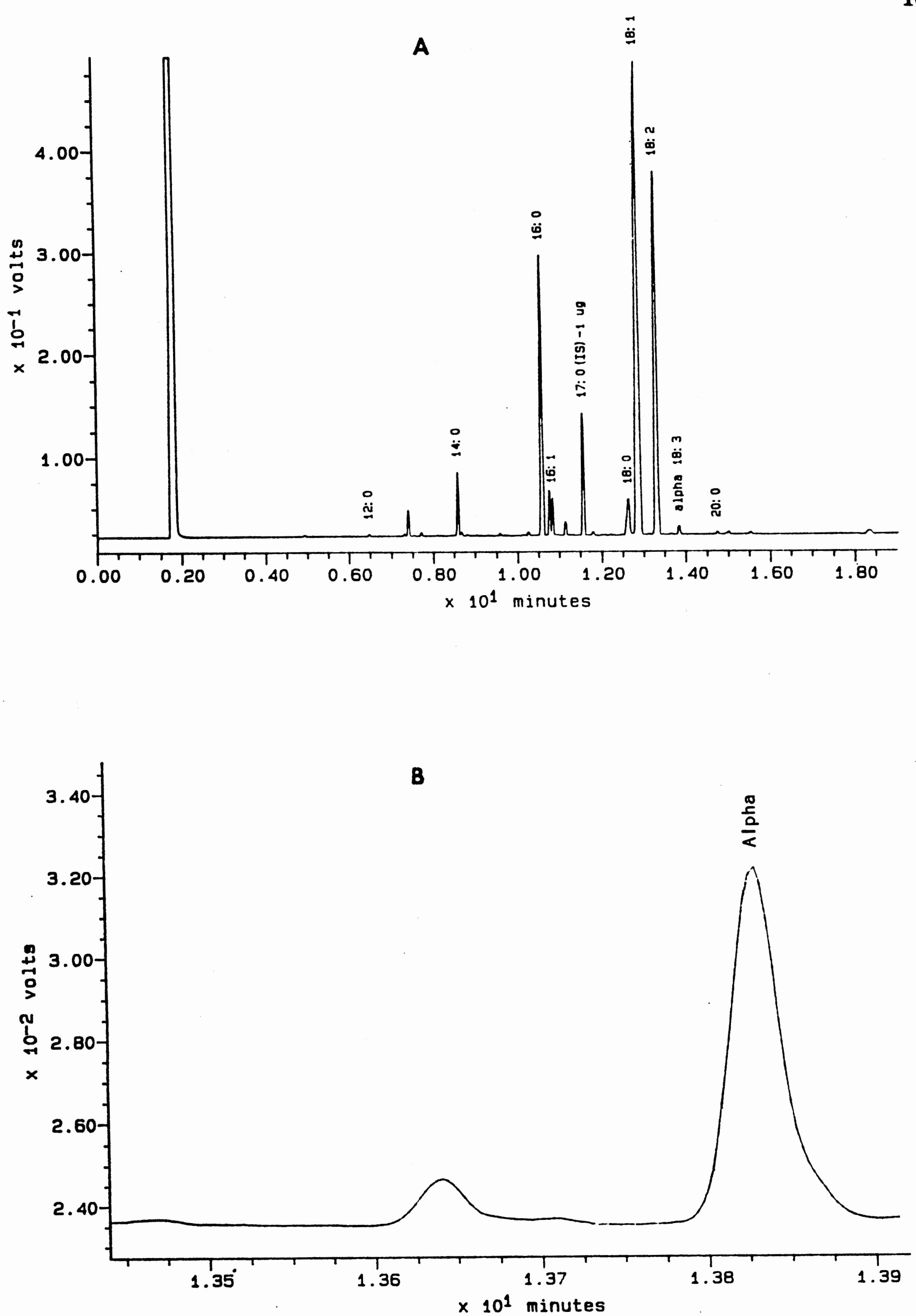
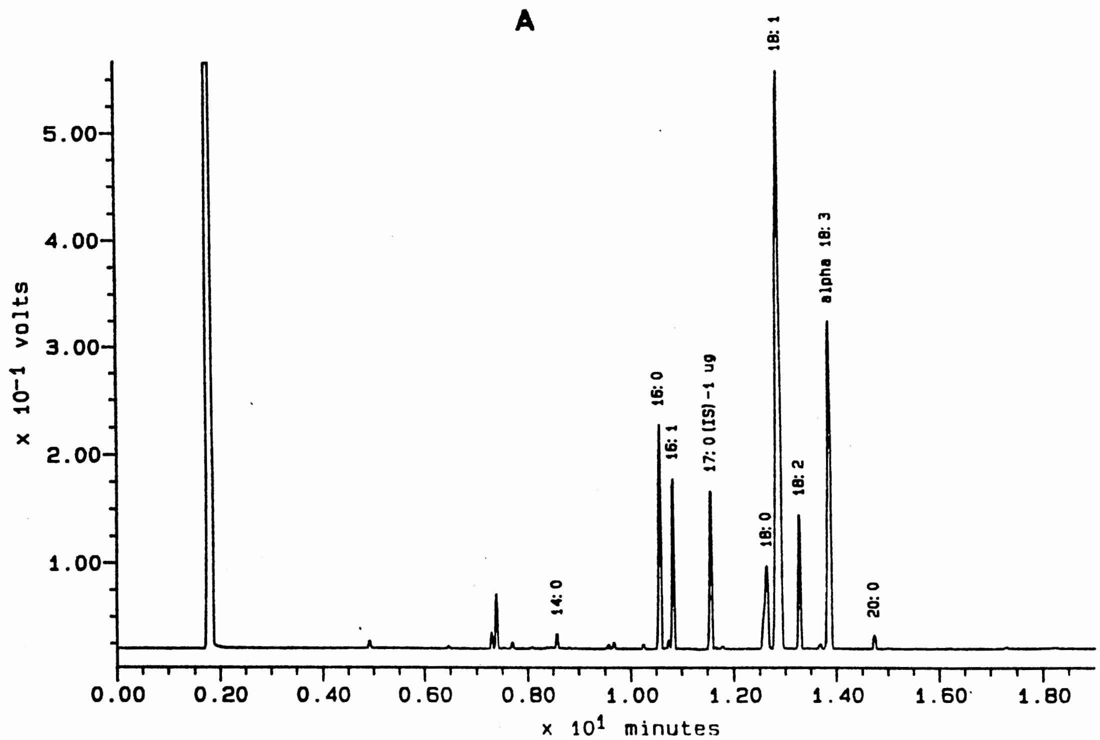


Figure 28. GC Trace of the Total Lipid FAME of the Dark Mealworm (Tenebrio abscurus) (A) and the 18:3 Area of that Trace (B)



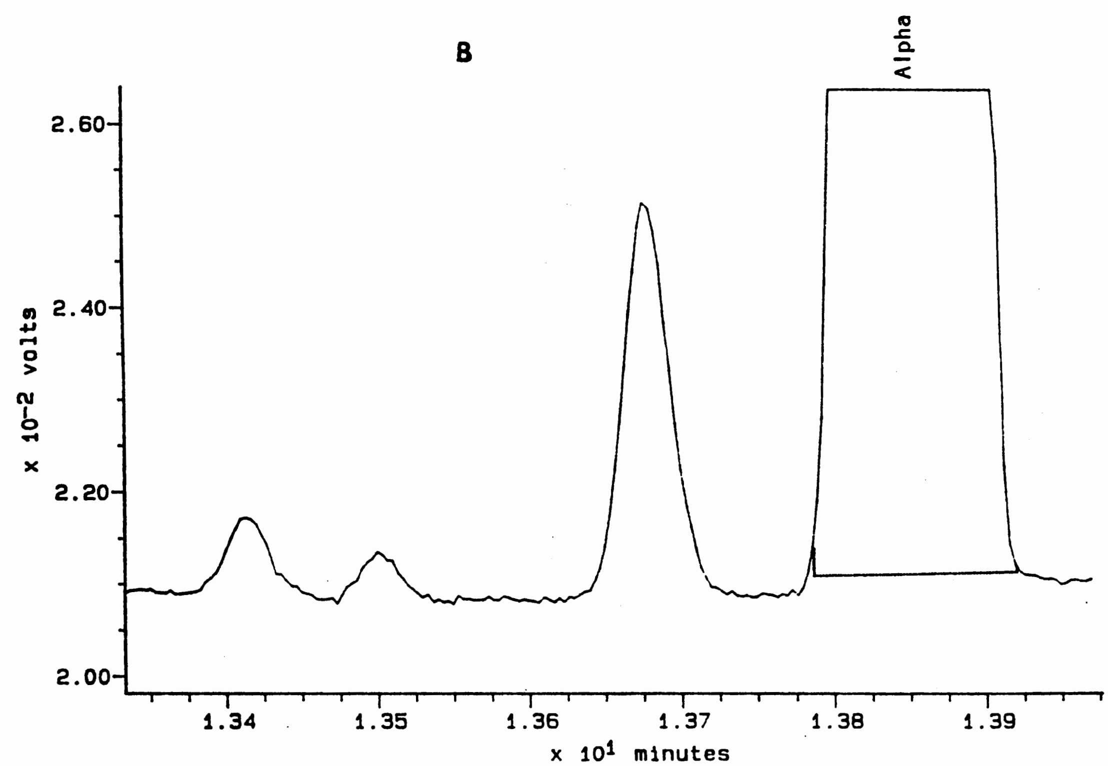


Figure 29. GC Trace of the Total Lipid FAME of the Alfalfa Weevil (Hypera postica) (A) and the 18:3 Area of that Trace (B)

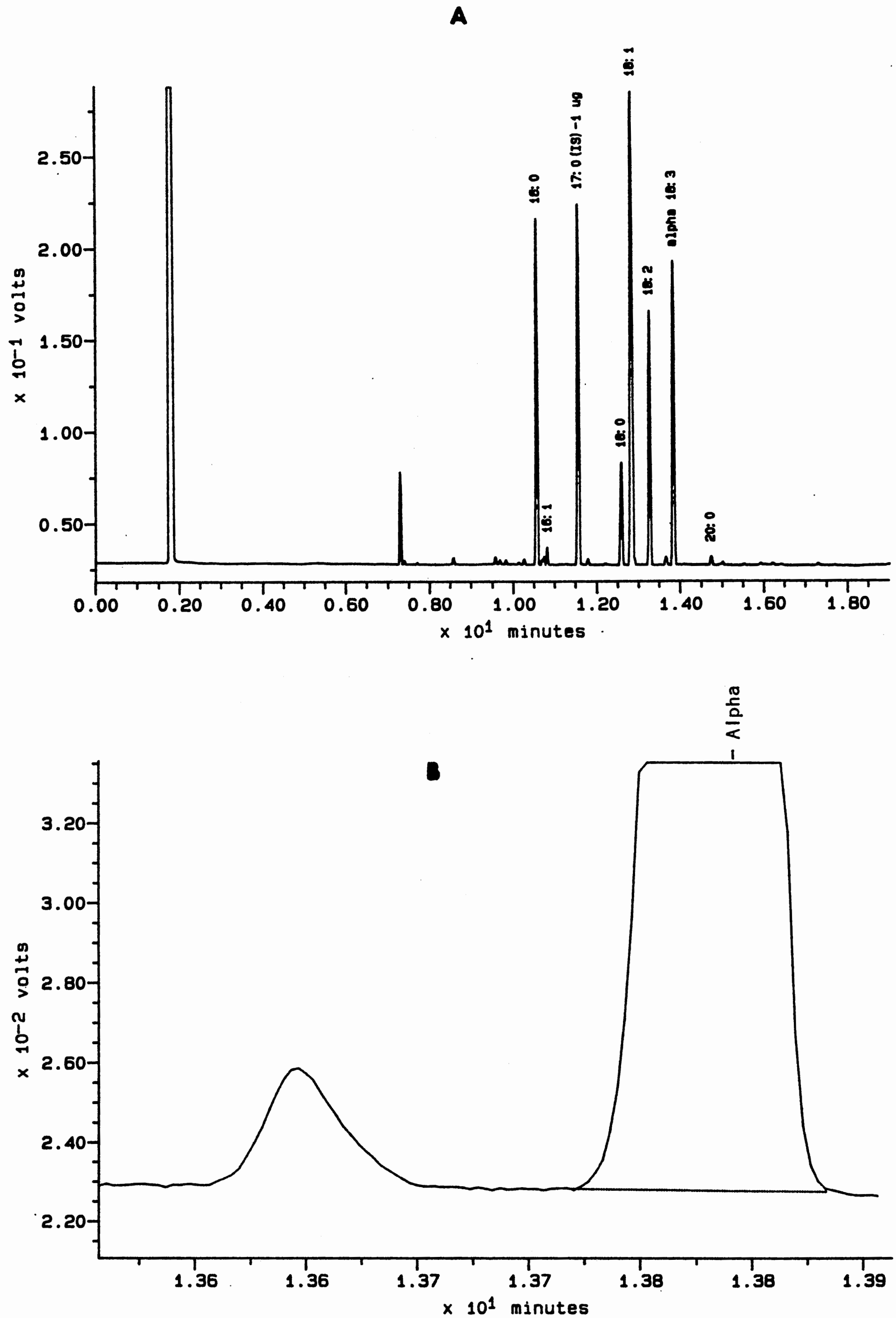


Figure 30. GC Trace of the Total Lipid FAME of the Spotted Cucumber Beetle (Diabrotica undecimpunctata howardi) (garden) (A) and the 18:3 Area of that Trace (B)

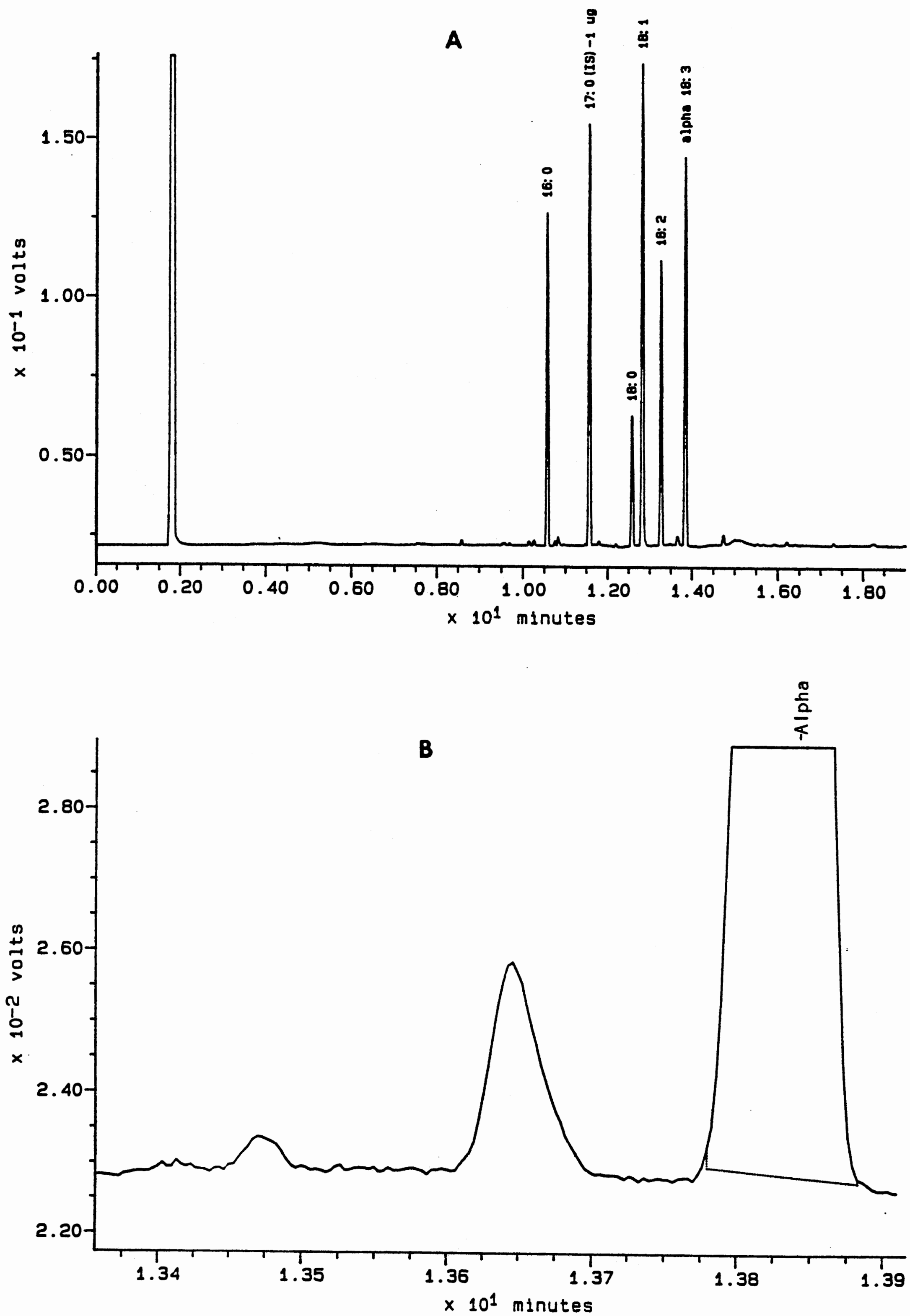


Figure 31. GC Trace of the Total Lipid FAME of the Spotted Cucumber Beetle (Diabrotica undecimpunctata howardi) (alfalfa) (A) and the 18:3 Area of that Trace (B)

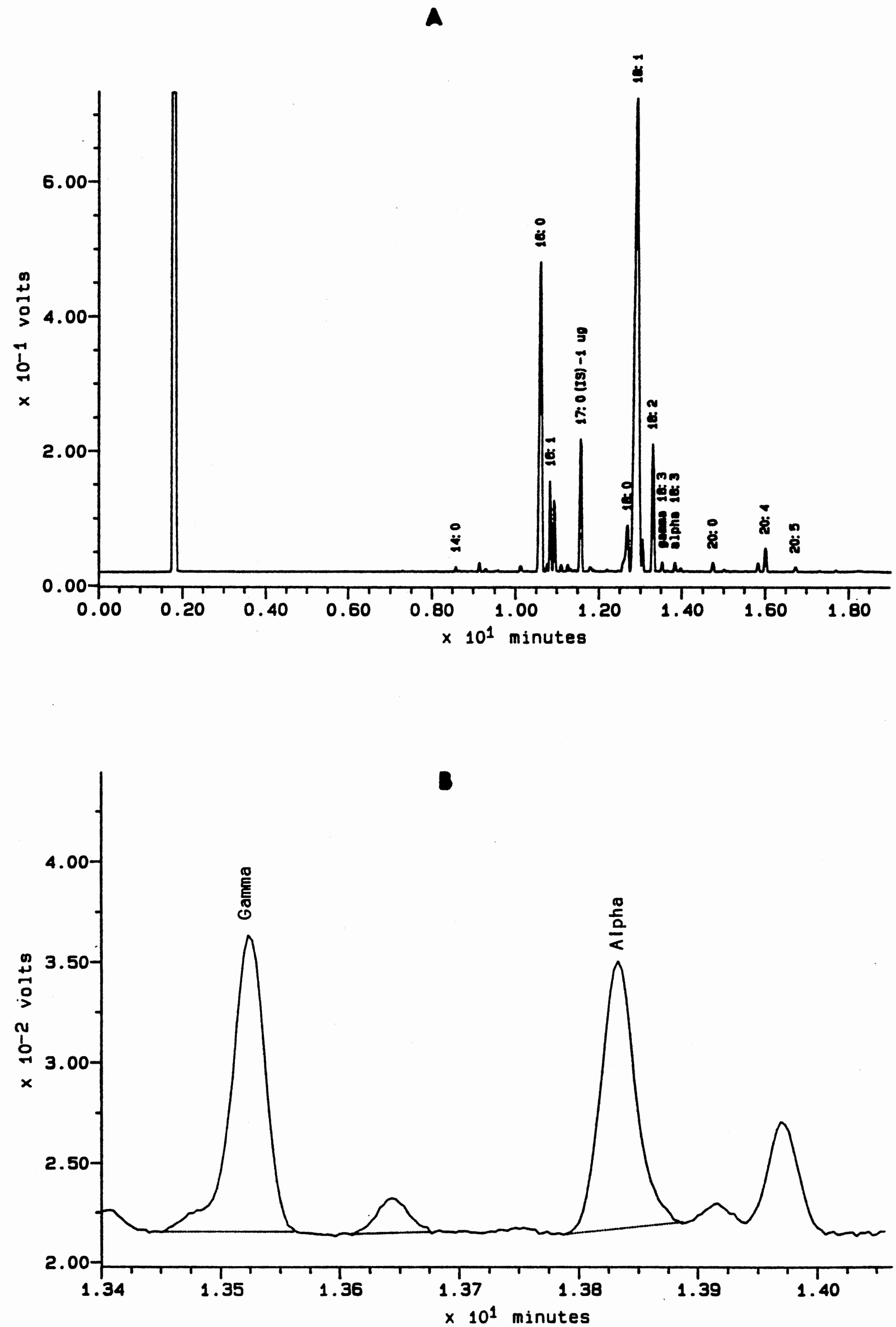
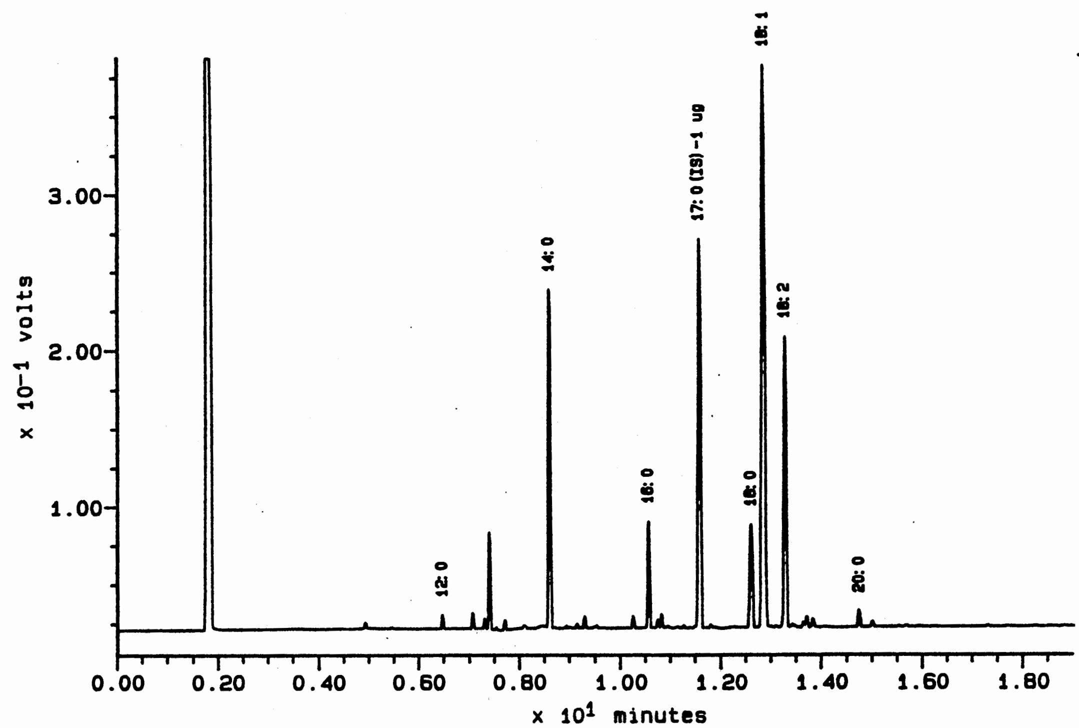


Figure 32. GC Trace of the Total Lipid FAME of the Southern Masked Chafer (Cyclocephala immaculata) (A) and the 18:3 Area of that Trace (B)

A



B

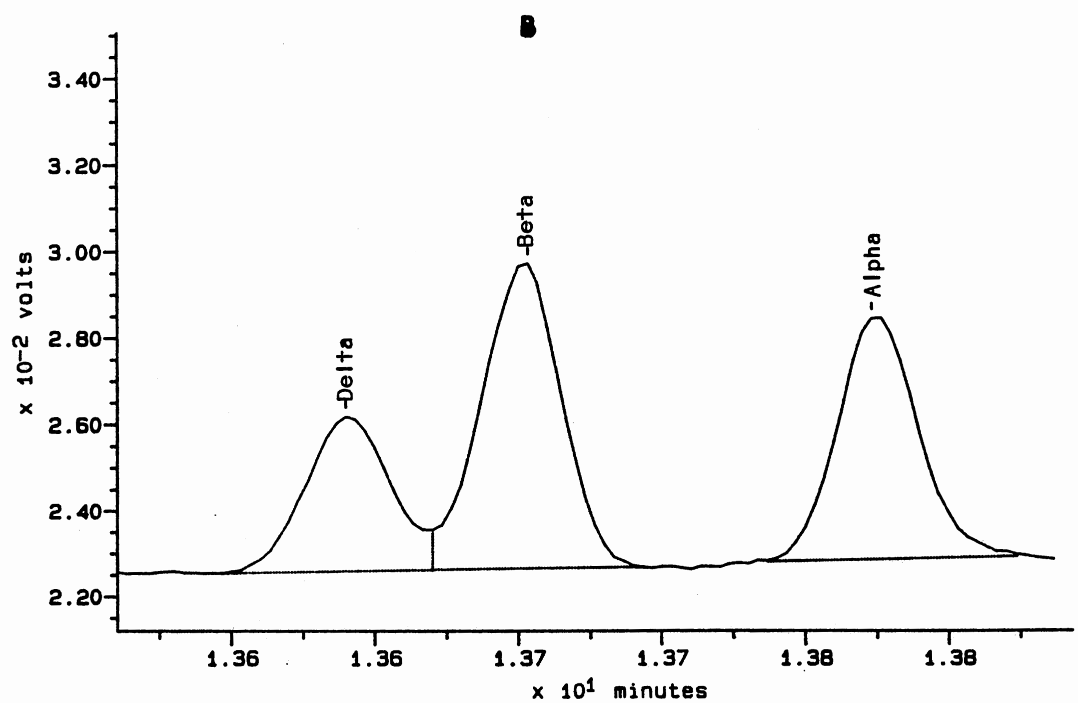
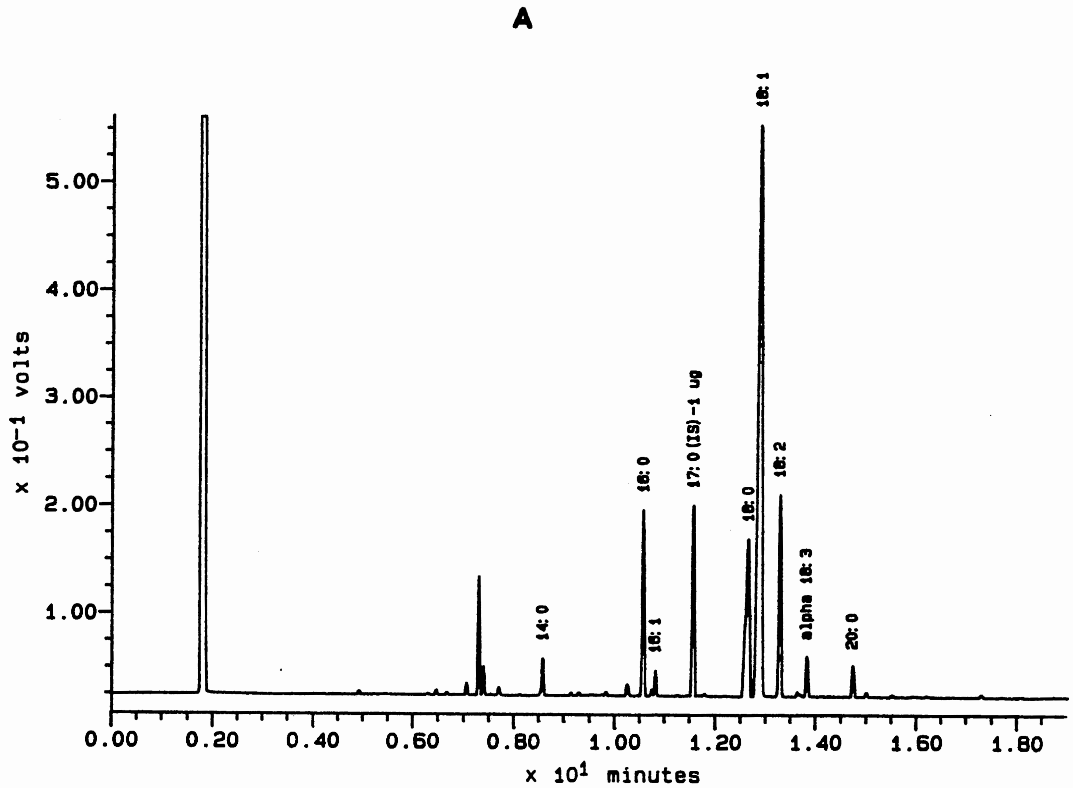


Figure 33. GC Trace of the Total Lipid FAME of the C7 Lady Beetle (Coccinella septempunctata) (A) and the 18:3 Area of that Trace (B)



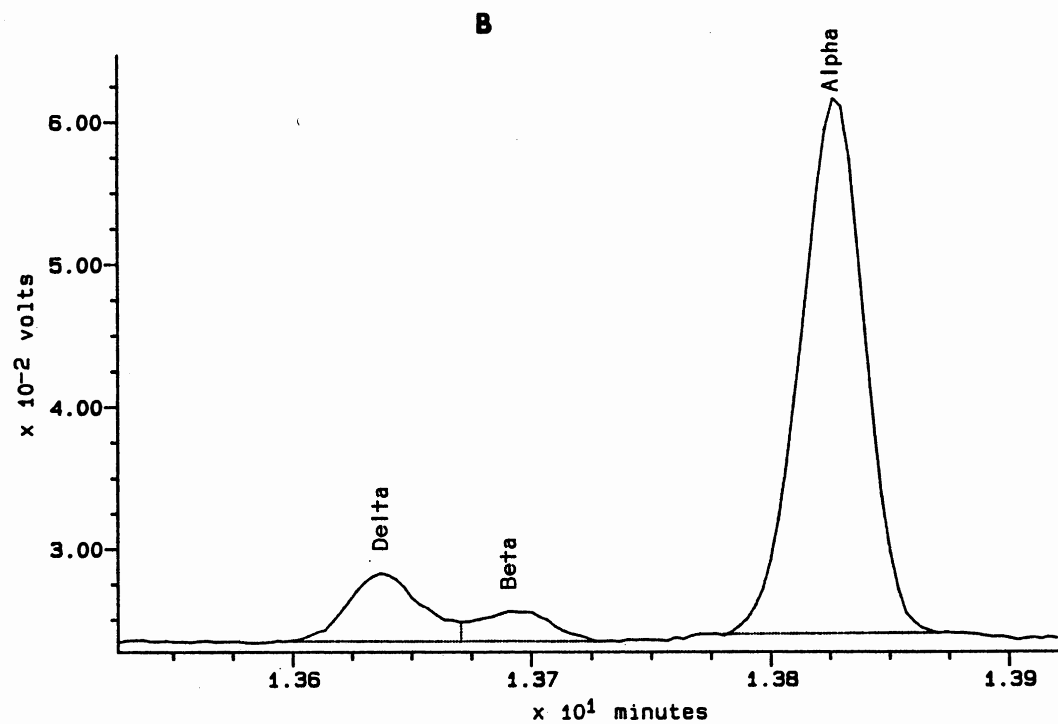


Figure 34. GC Trace of the Total Lipid FAME of the Spotted Lady Beetle (Coleomegilla maculata) (A) and the 18:3 Area of that Trace (B)

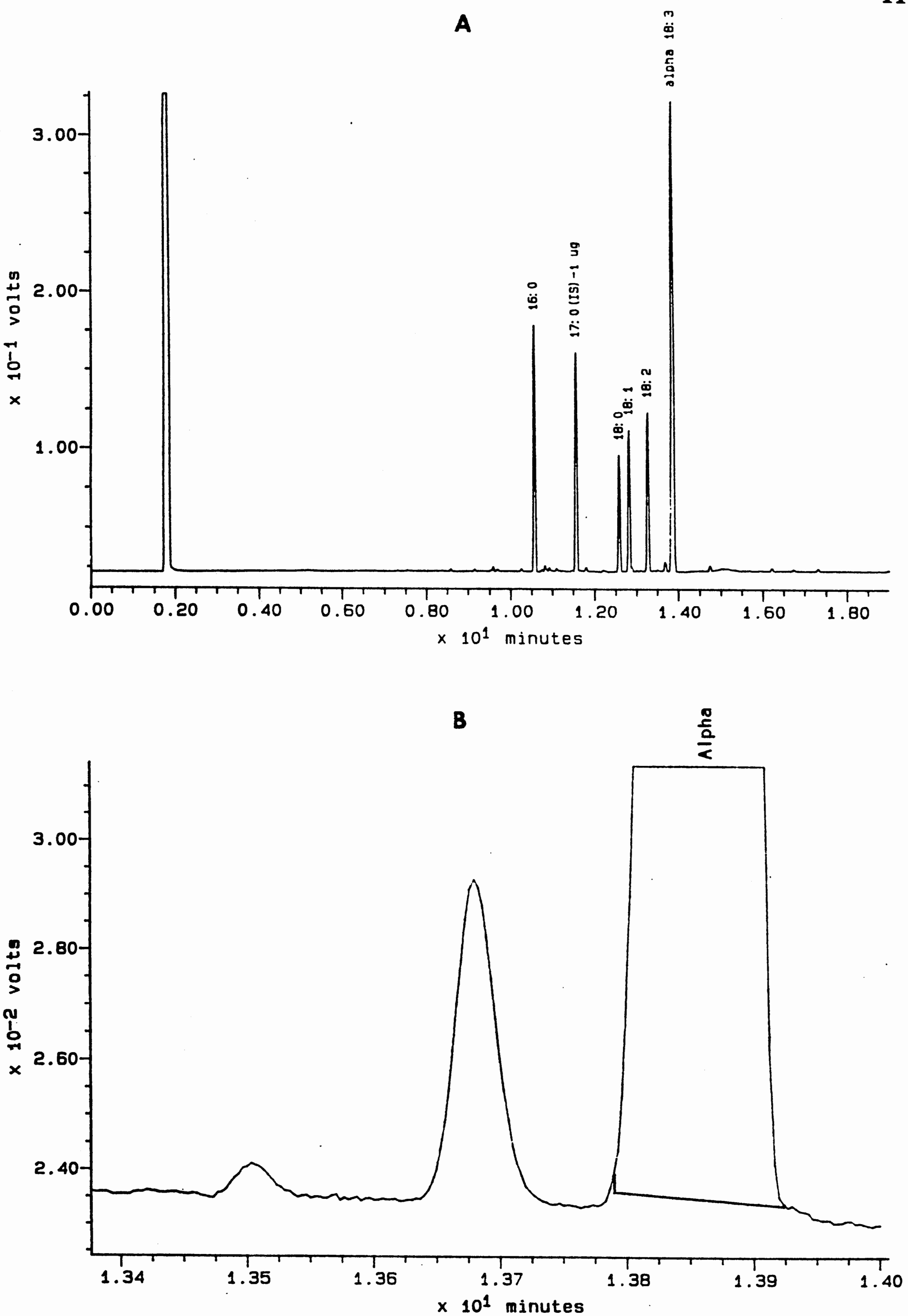


Figure 35. GC Trace of the Total Lipid FAME of the Milkweed Tiger Moth (<u>Euchaetes egle</u>) (A) and the 18:3 Area of that Trace (B)

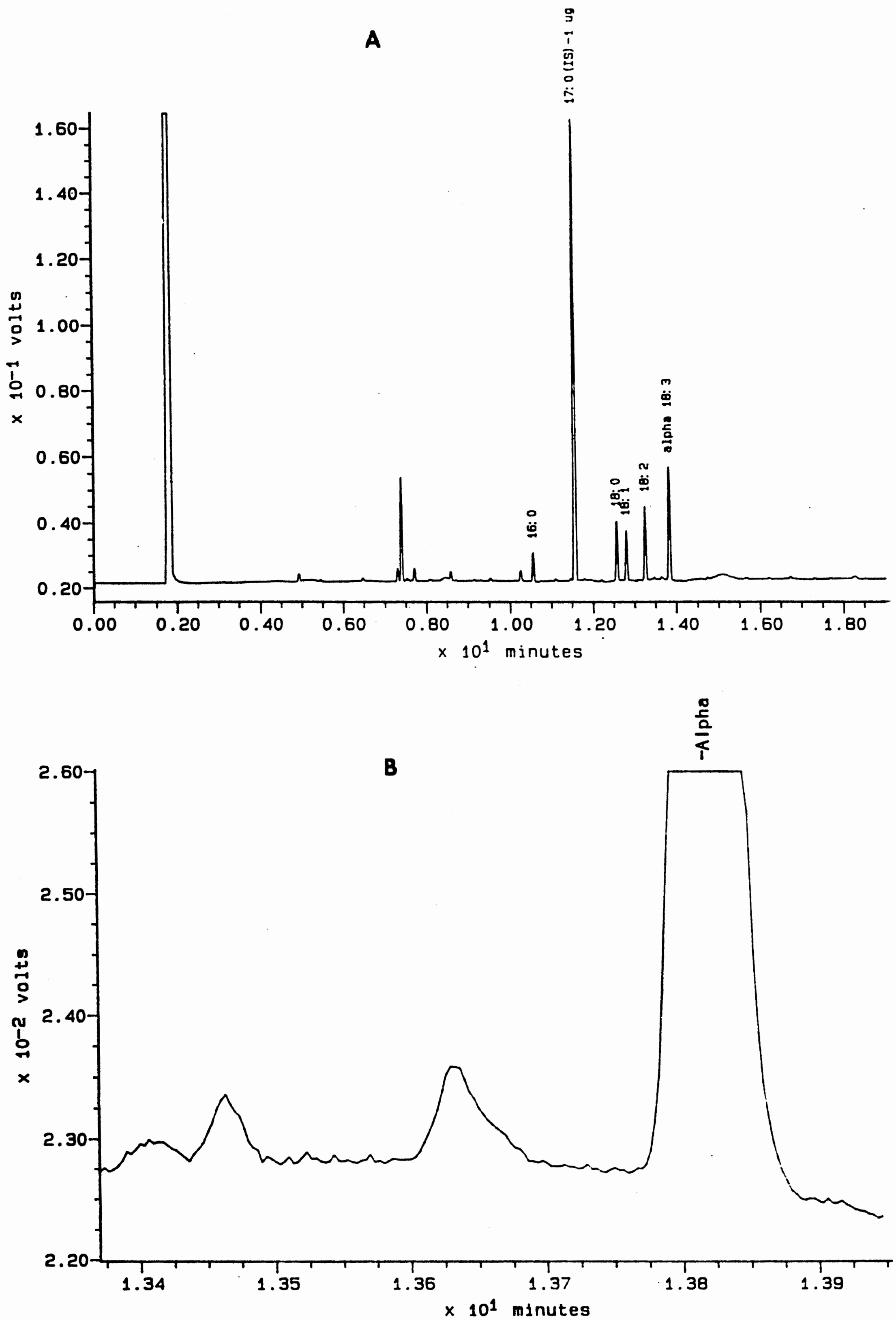
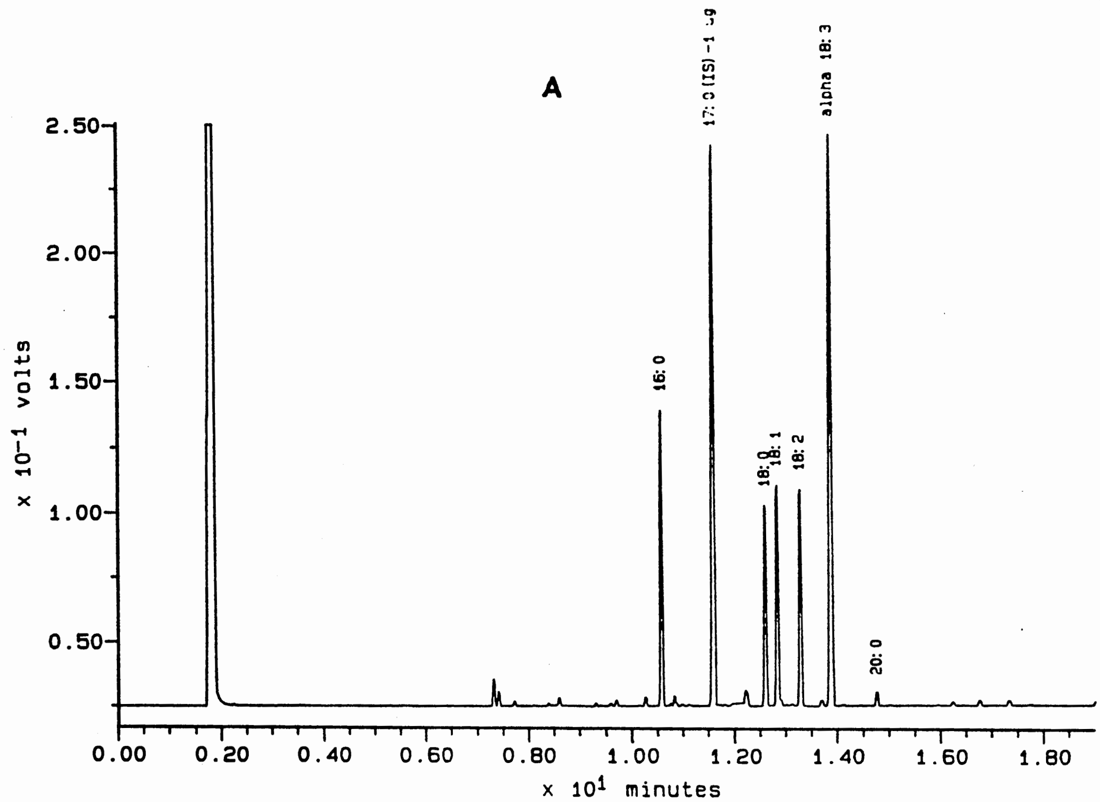


Figure 36. GC Trace of the Total Lipid FAME of the Milkweed Tiger Moth (Euchaetes egle) (starved) (A) and the 18:3 Area of that Trace (B)



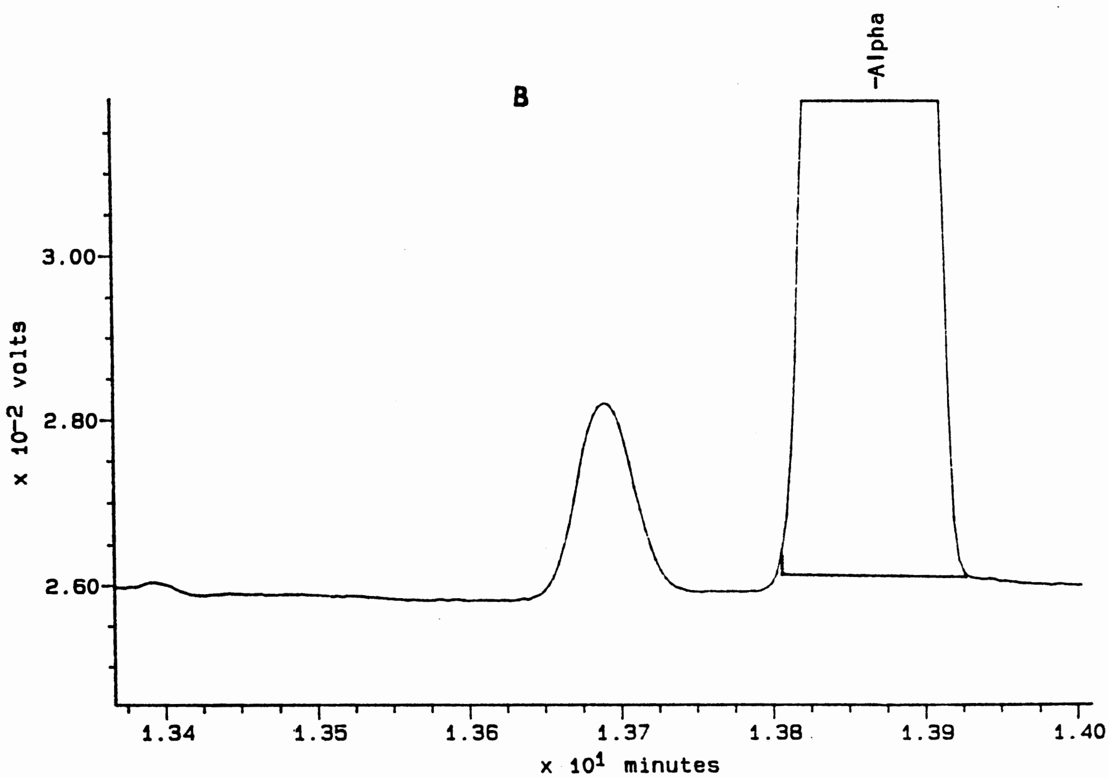


Figure 37. GC Trace of the Total Lipid FAME of the Fall Webworm (*Hyphantria cunea*) (River Birch) (A) and the 18:3 Area of that Trace (B)

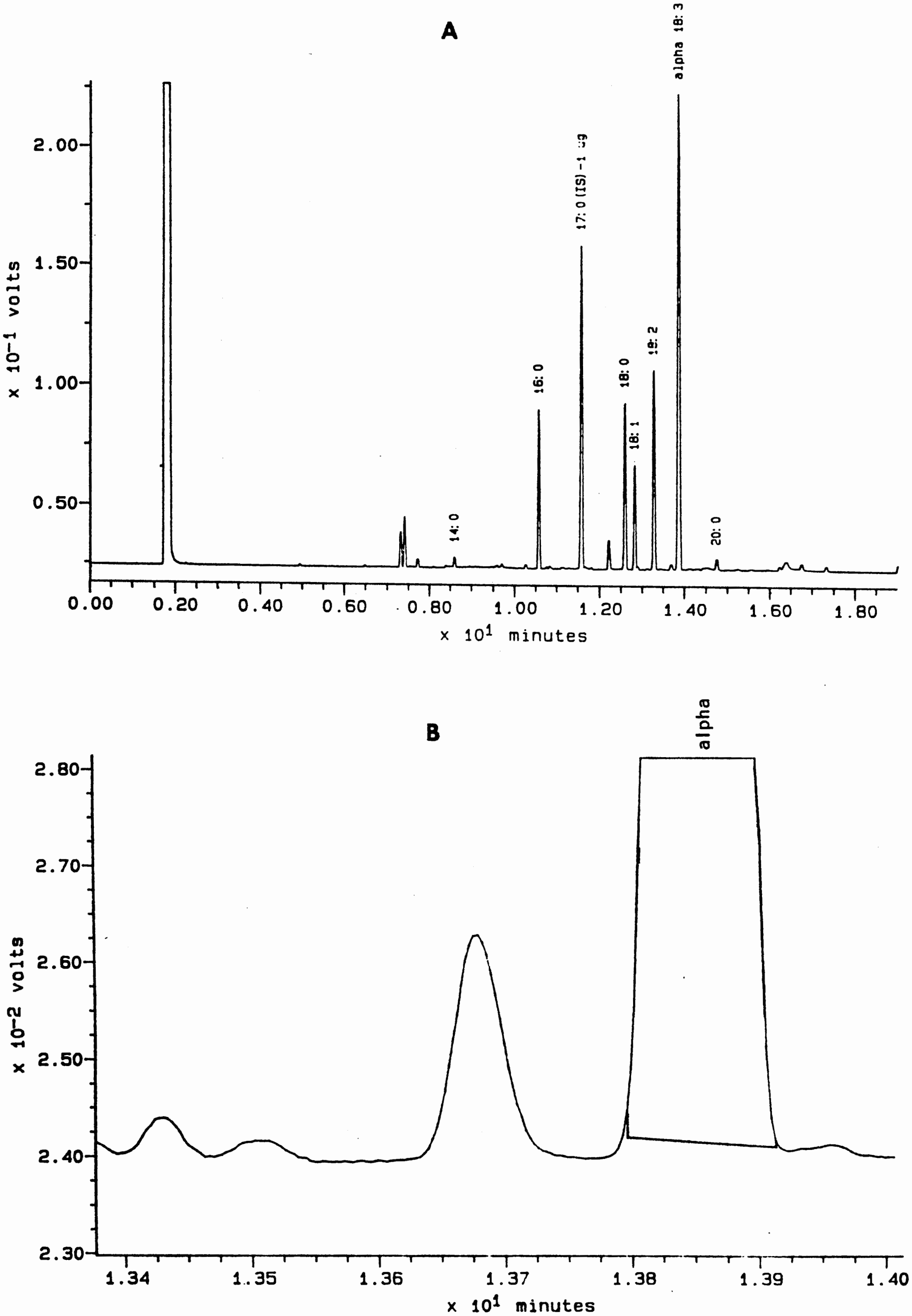


Figure 38. GC Trace of the Total Lipid FAME of the Fall Webworm (Hyphantria cunea) (Pecan) (A) and the 18:3 Area of that Trace (B)

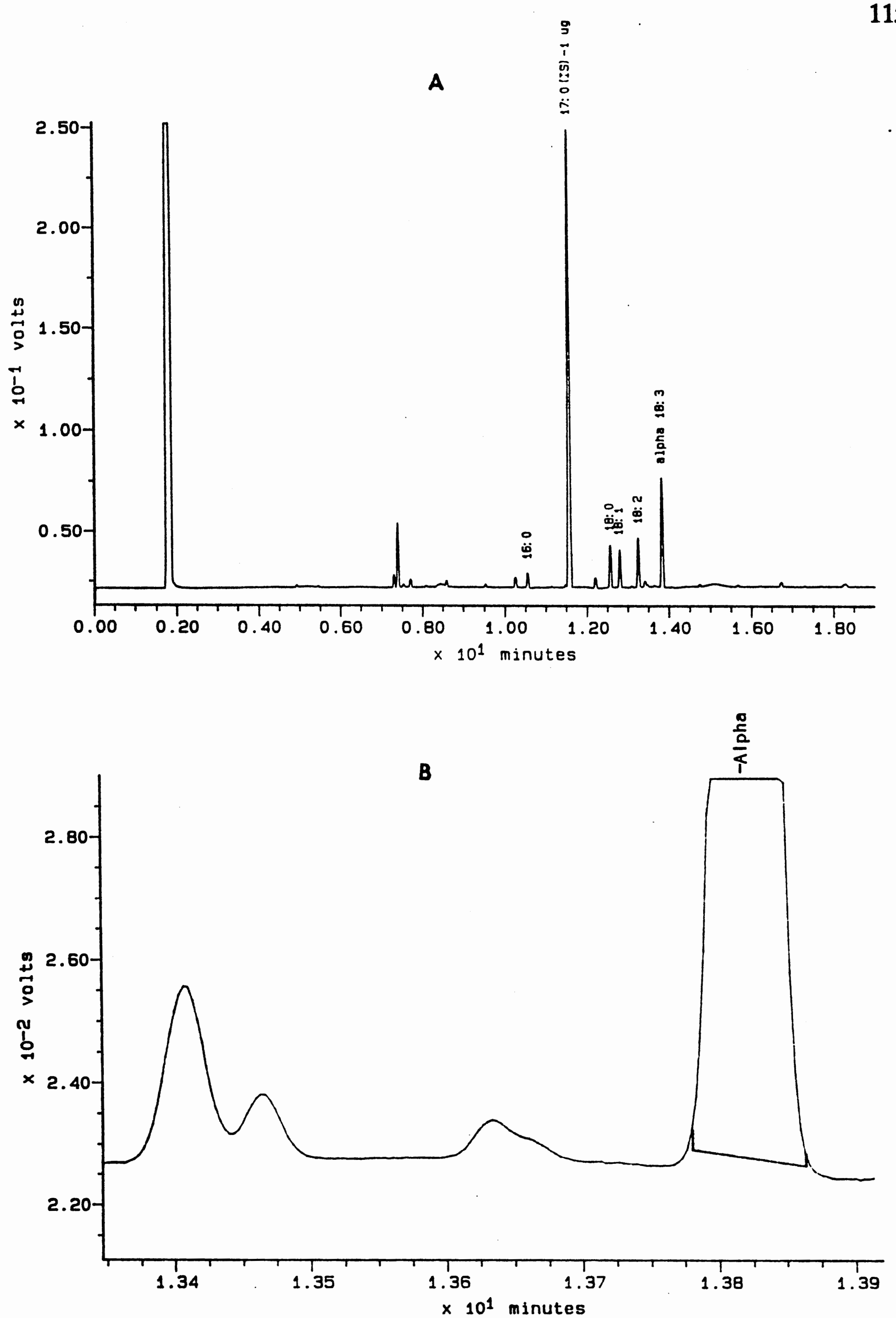


Figure 39. GC Trace of the Total Lipid FAME of the Fall Webworm (Hyphantria cunea) (starved) (A) and the 18:3 Area of that Trace (B)

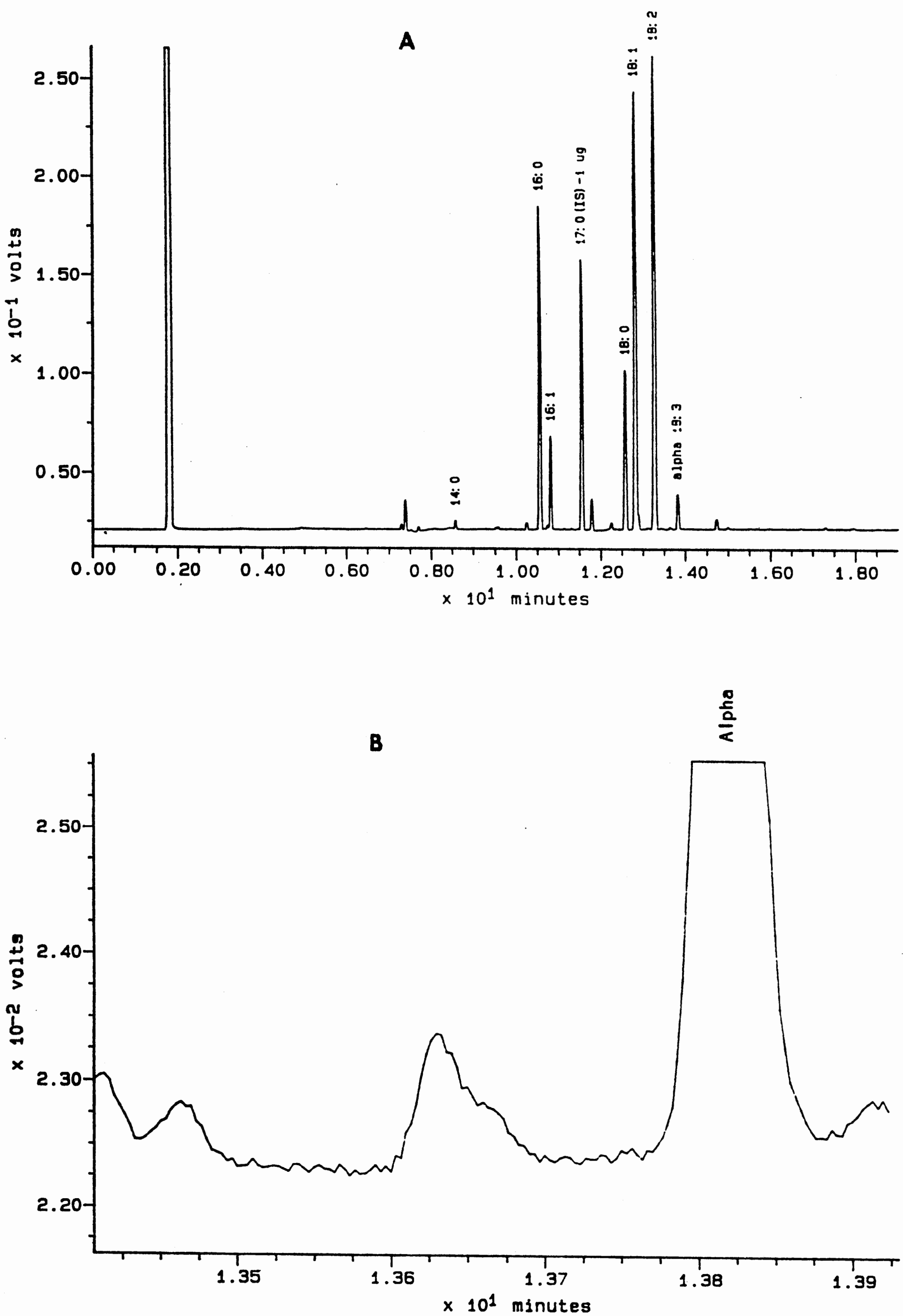


Figure 40. GC Trace of the Total Lipid FAME of the Tobacco Hornworm (Manduca sexa) (A) and the 18:3 Area of that Trace (B)

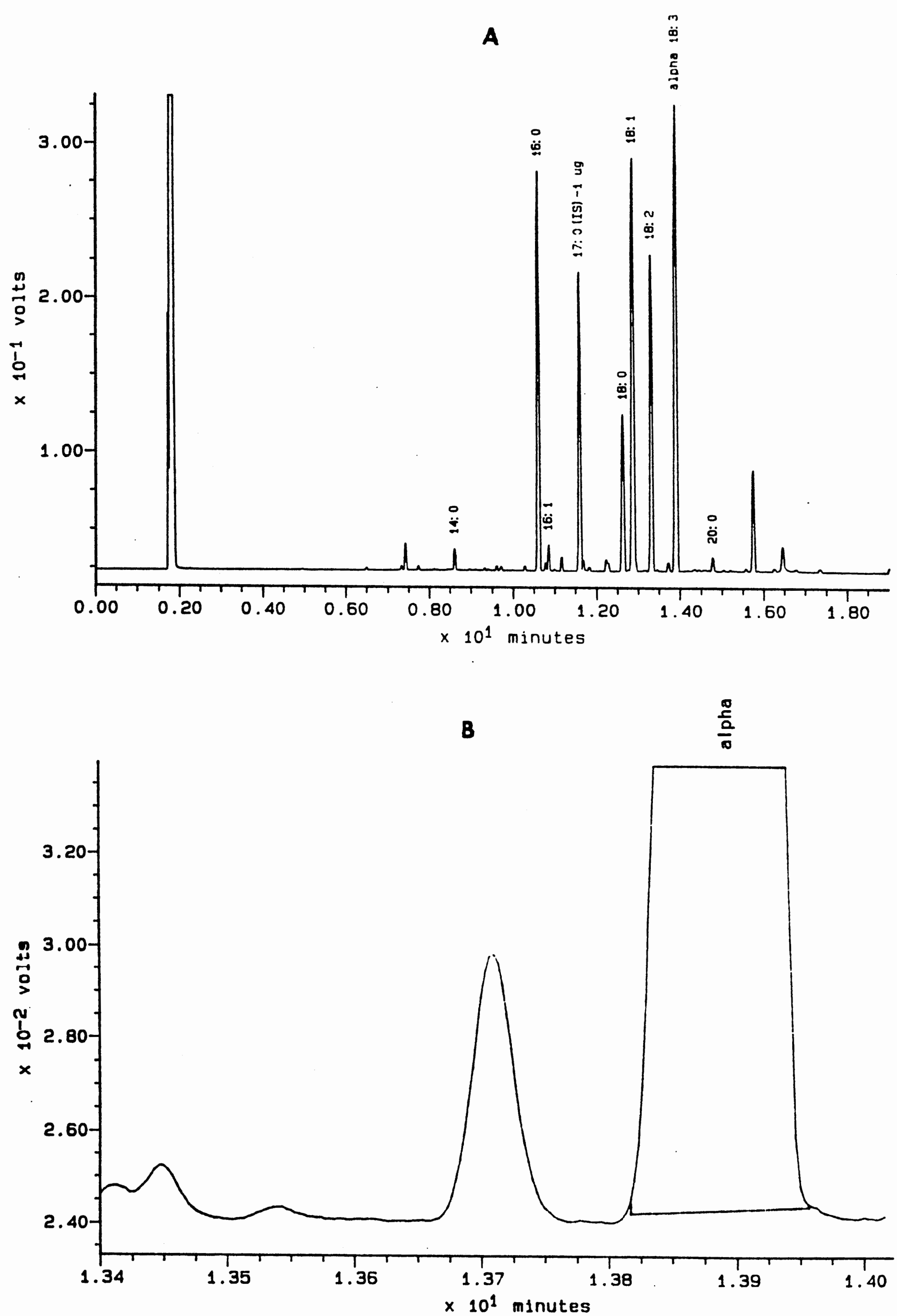


Figure 41. GC Trace of the Total Lipid FAME of the Bagworm (Thyridioteryx ephemeraeformis) (A) and the 18:3 Area of that Trace (B)

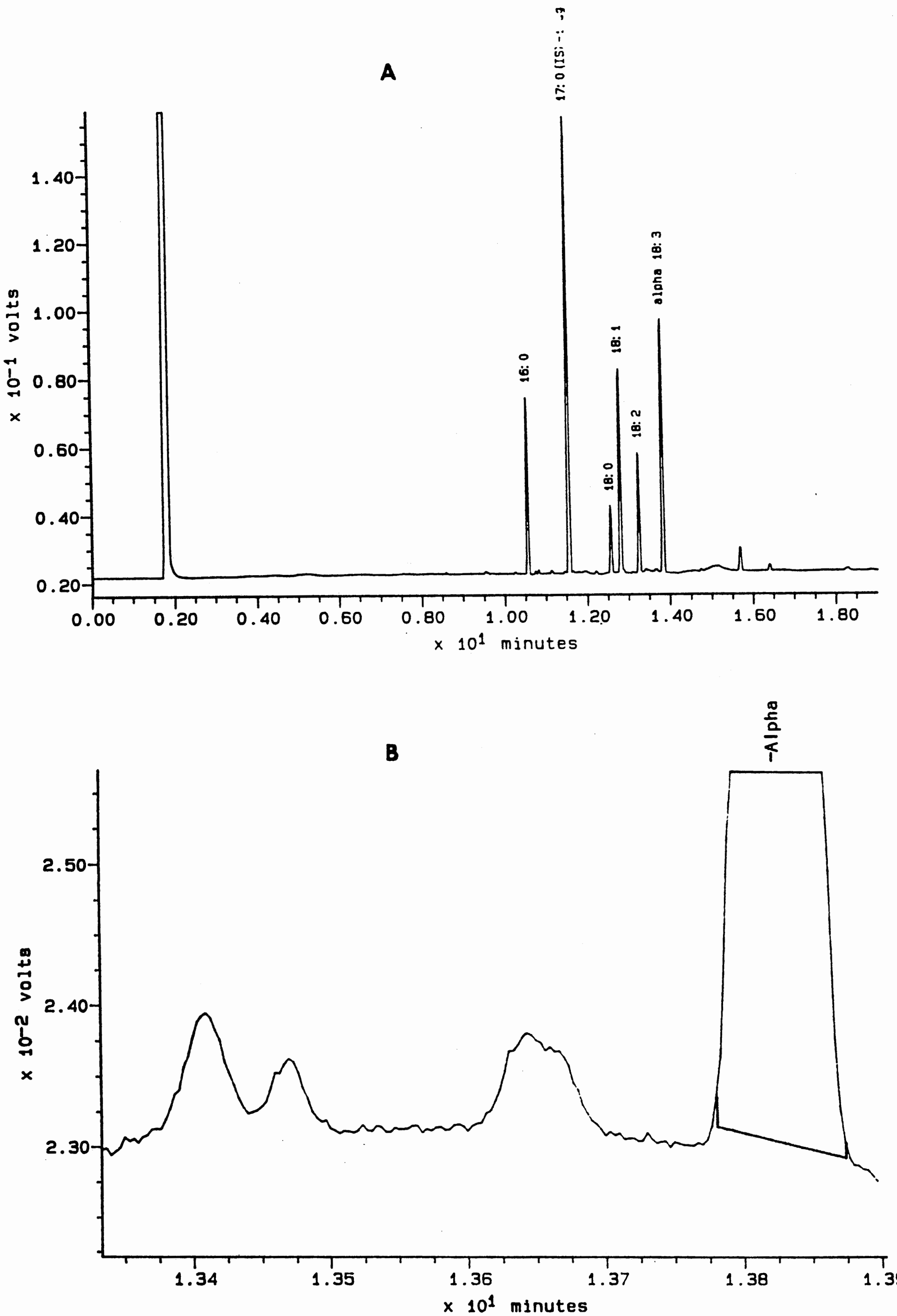


Figure 42. GC Trace of the Total Lipid FAME of the Bagworm hemolymph (Thyridioteryx ephemeraeformis) (A) and the 18:3 Area of that Trace (B)

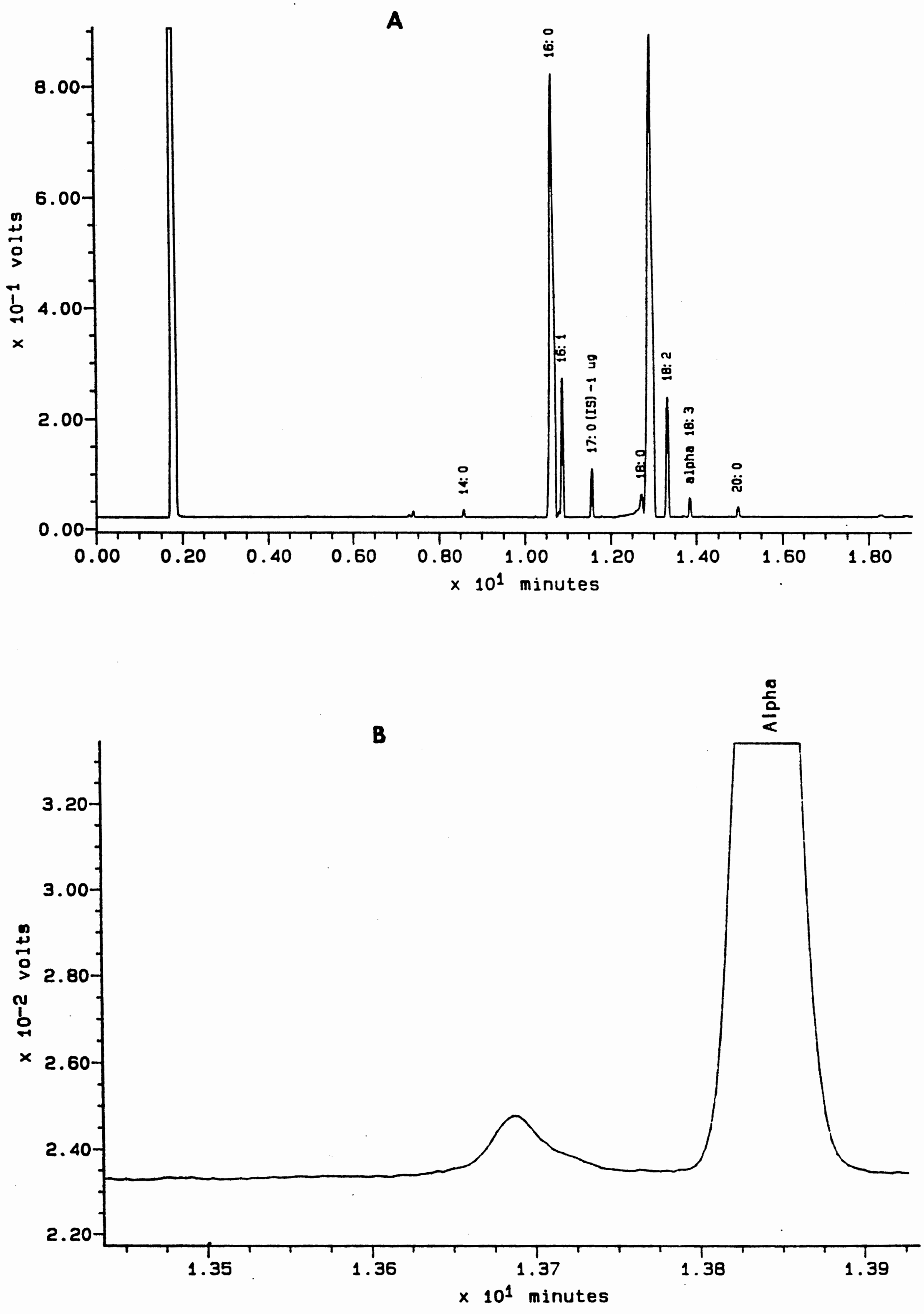
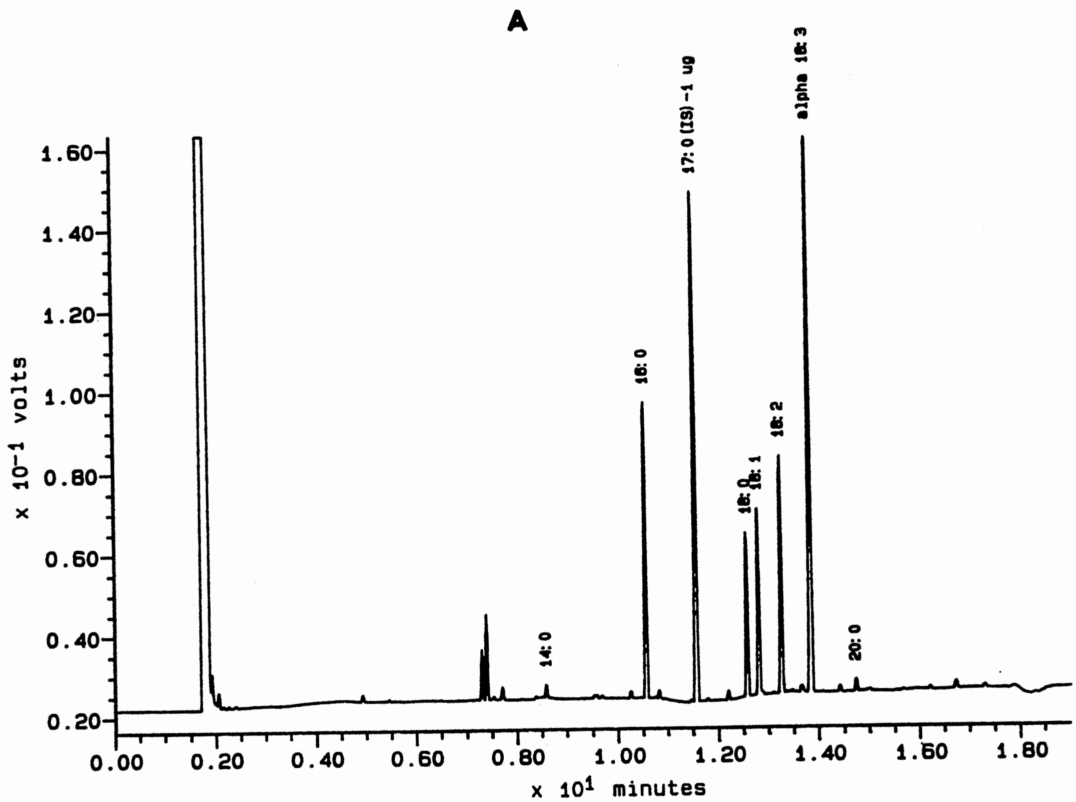


Figure 43. GC Trace of the Total Lipid FAME of the Greater Wax Moth (Galleria mellonella) (A) and the 18:3 Area of that Trace (B)



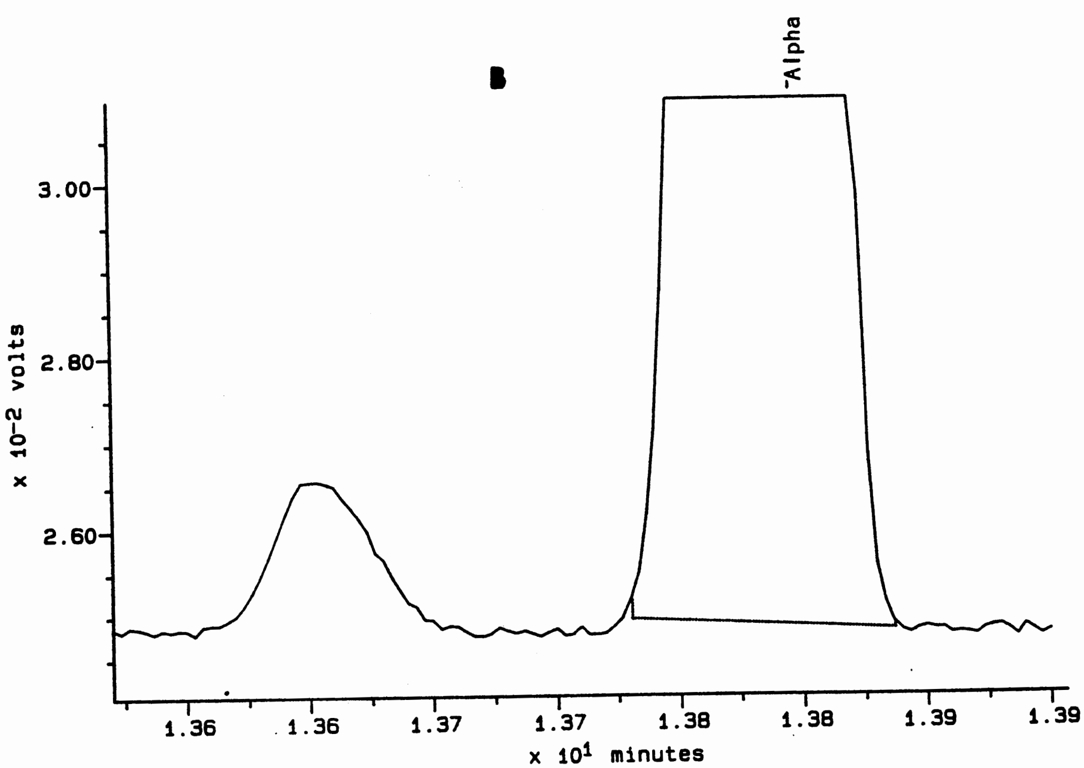
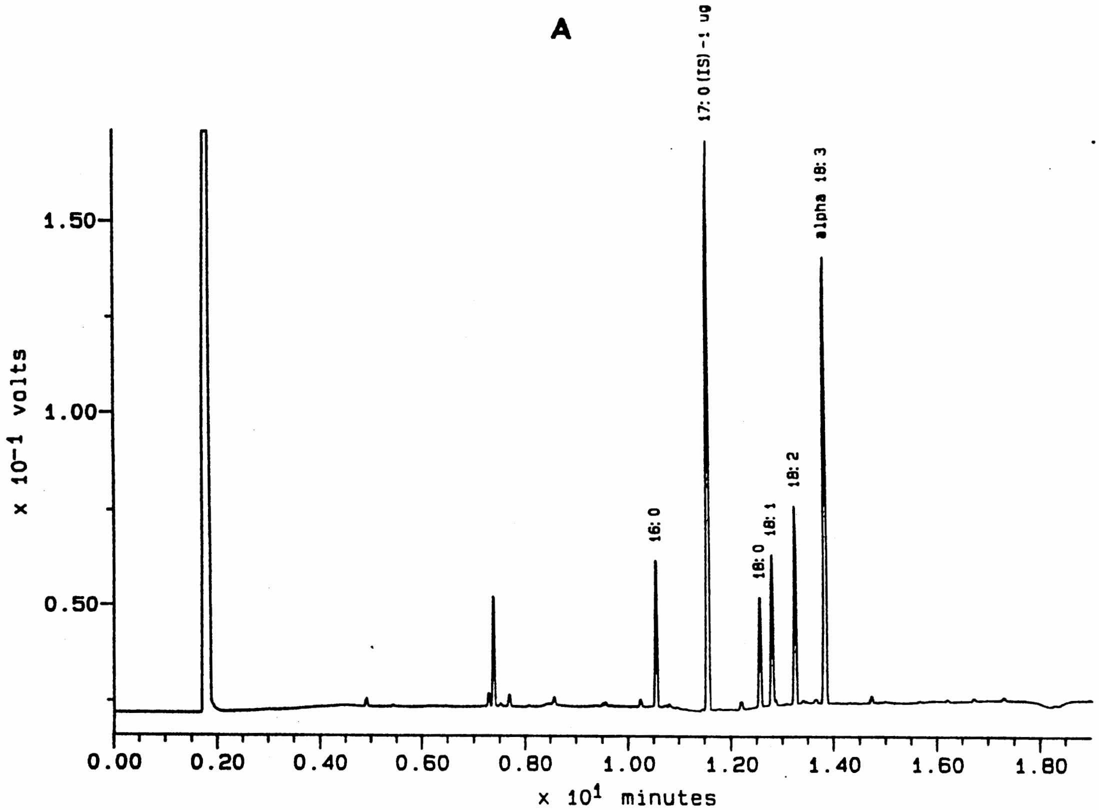


Figure 44. GC Trace of the Total Lipid FAME of the Alfalfa Leafworm (Heliothis zea) (A) and the 18:3 Area of that Trace (B)



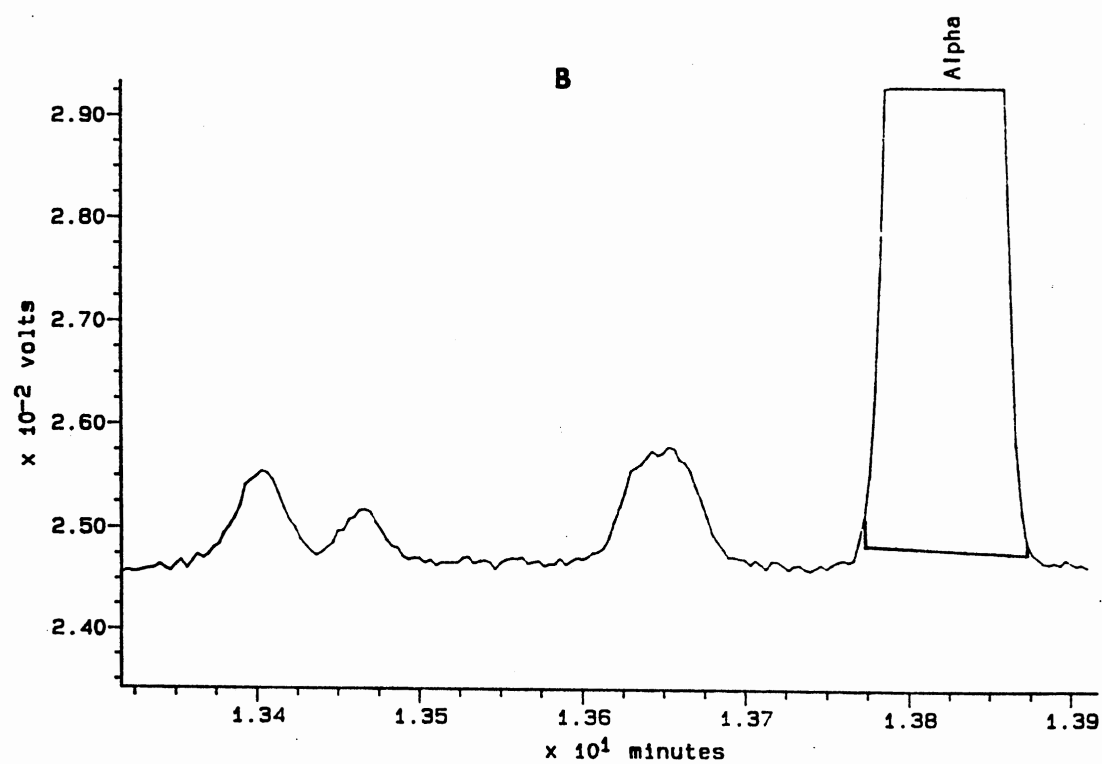


Figure 45. GC Trace of the Total Lipid FAME of the Yellow-Stripped Armyworm (Spodoptera ornithogalli) (A) and the 18:3 Area of that Trace (B)

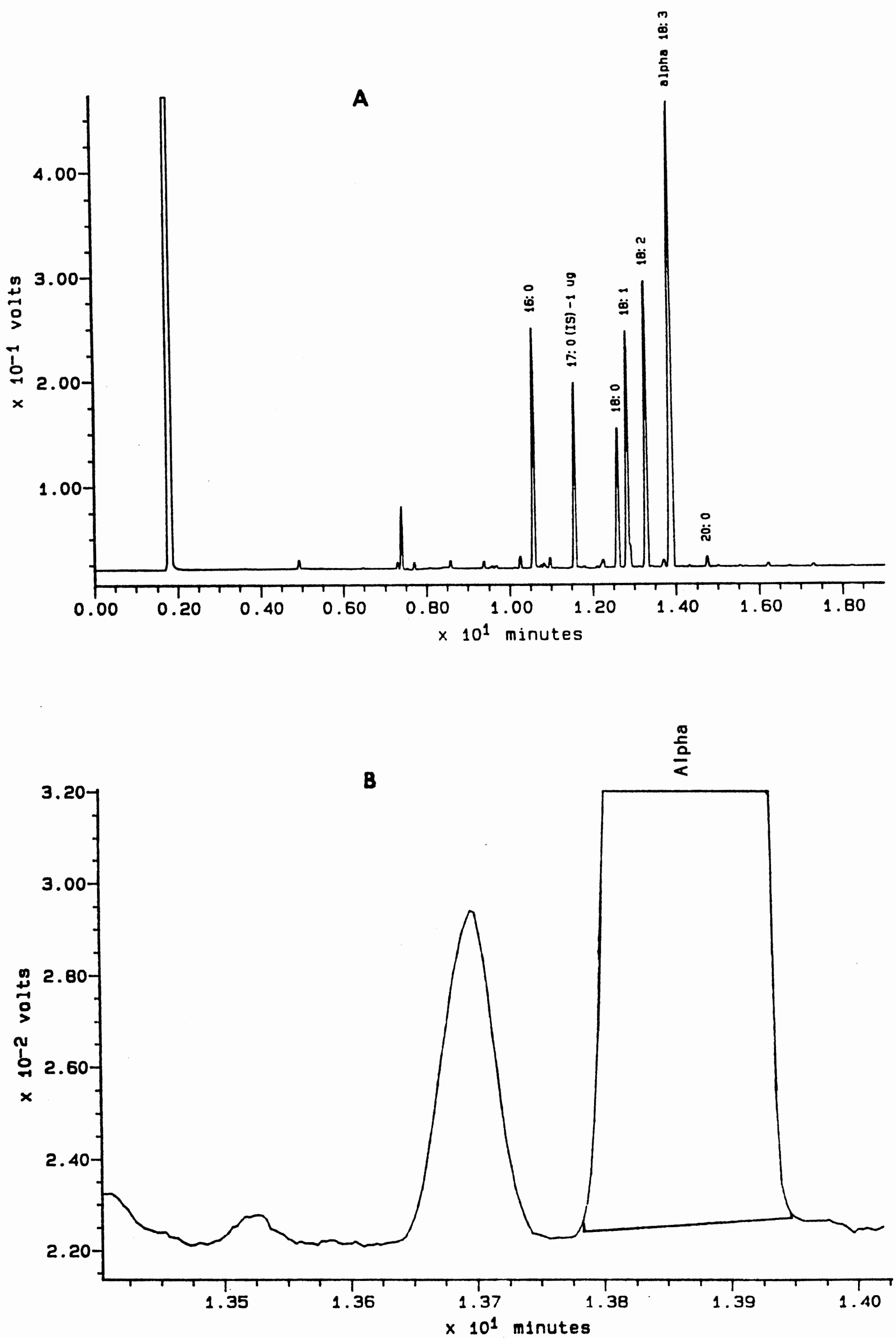


Figure 46. GC Trace of the Total Lipid FAME of the White-lined Sphinx (<u>Hyles lineata</u>) (A) and the 18:3 Area of that Trace (B)

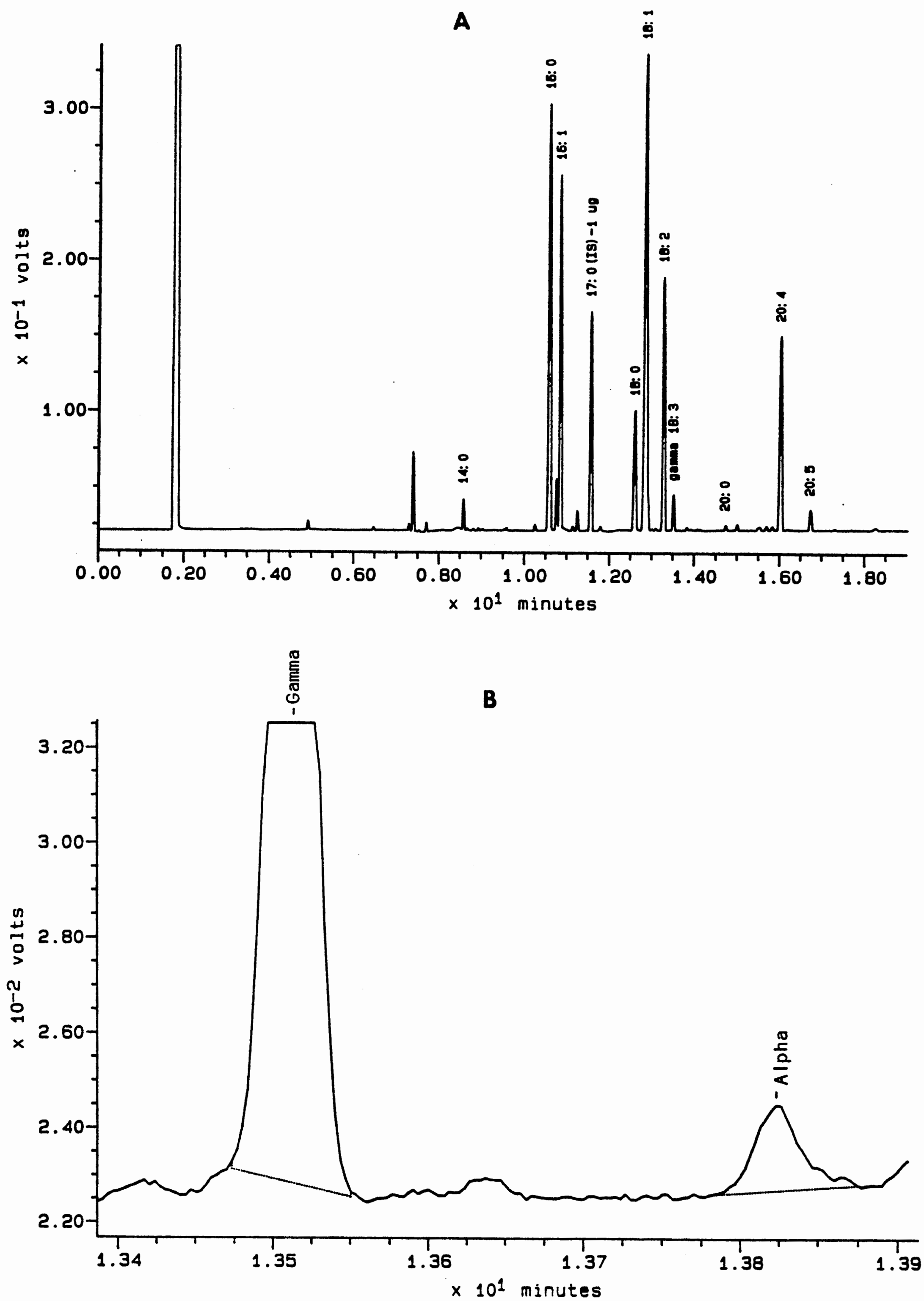
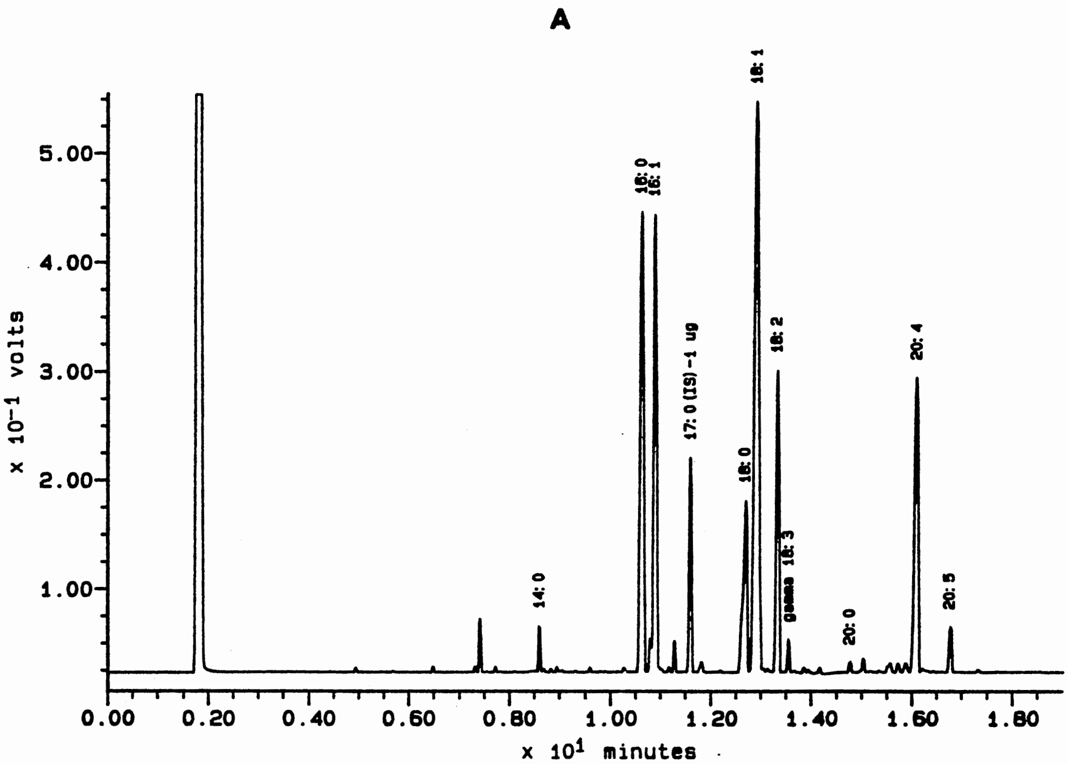


Figure 47. GC Trace of the Total Lipid FAME of the Blow Fly (Sarcophaga bullata) (A) and the 18:3 Area of that Trace (B)



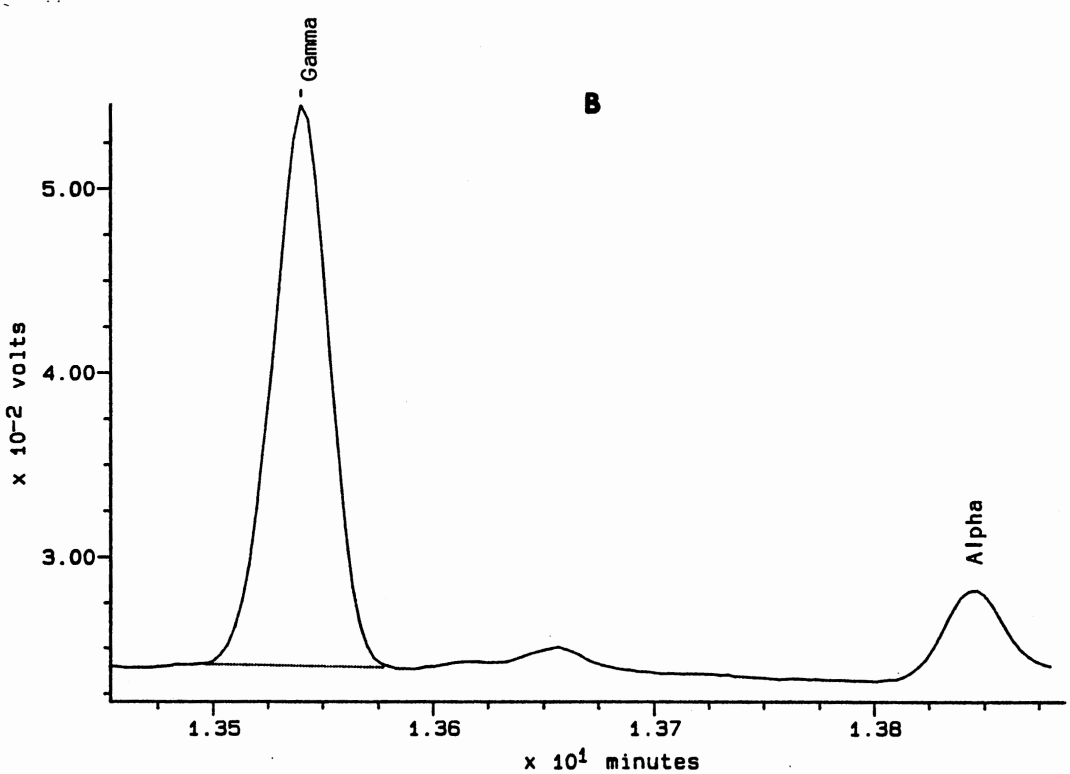


Figure 48. GC Trace of the Total Lipid FAME of the Blow Fly larvae (Sarcophaga bullata) (A) and the 18:3 Area of that Trace (B)

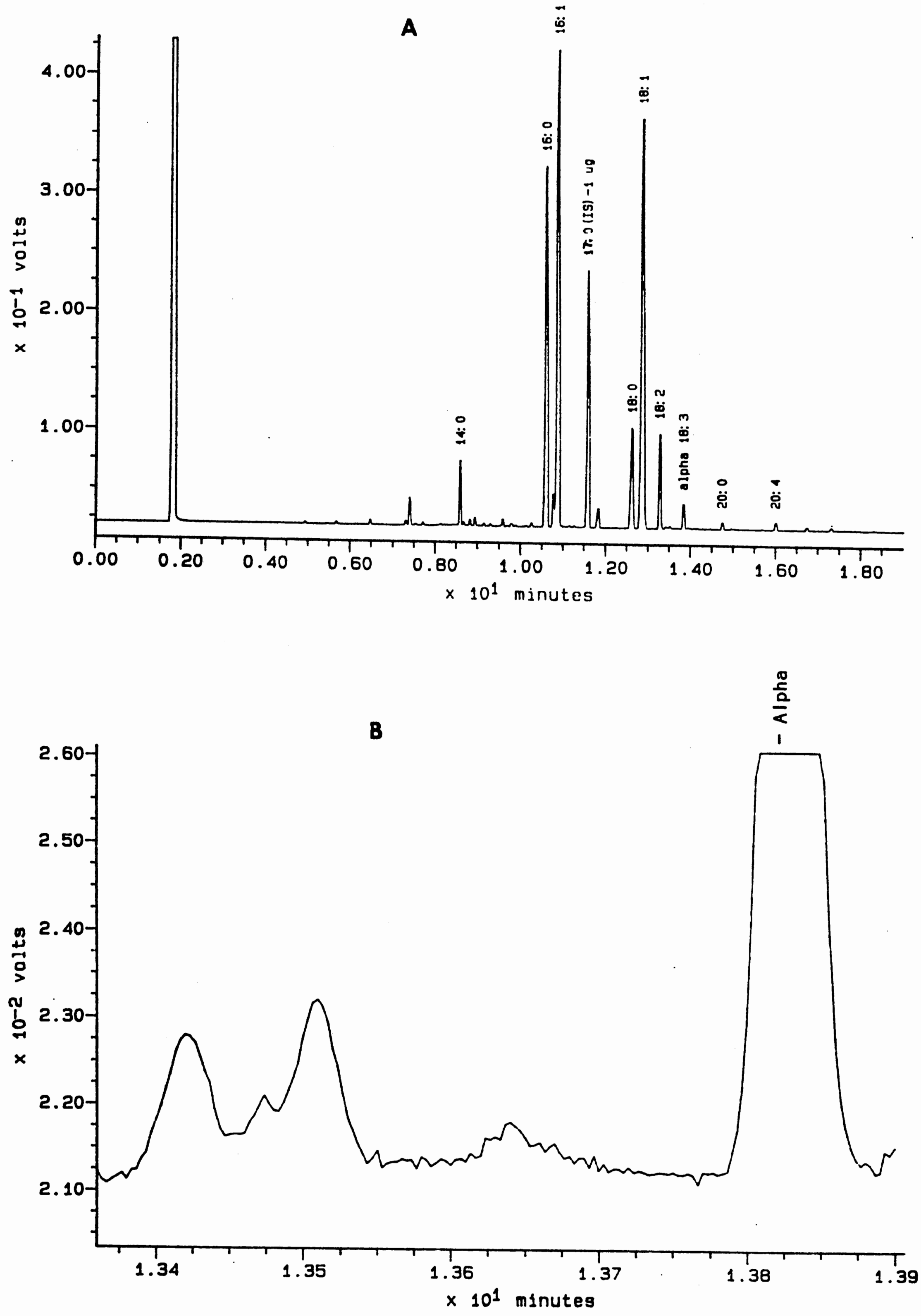


Figure 49. GC Trace of the Total Lipid FAME of the House Fly male (Musca domestica) (A) and the 18:3 Area of that Trace (B)

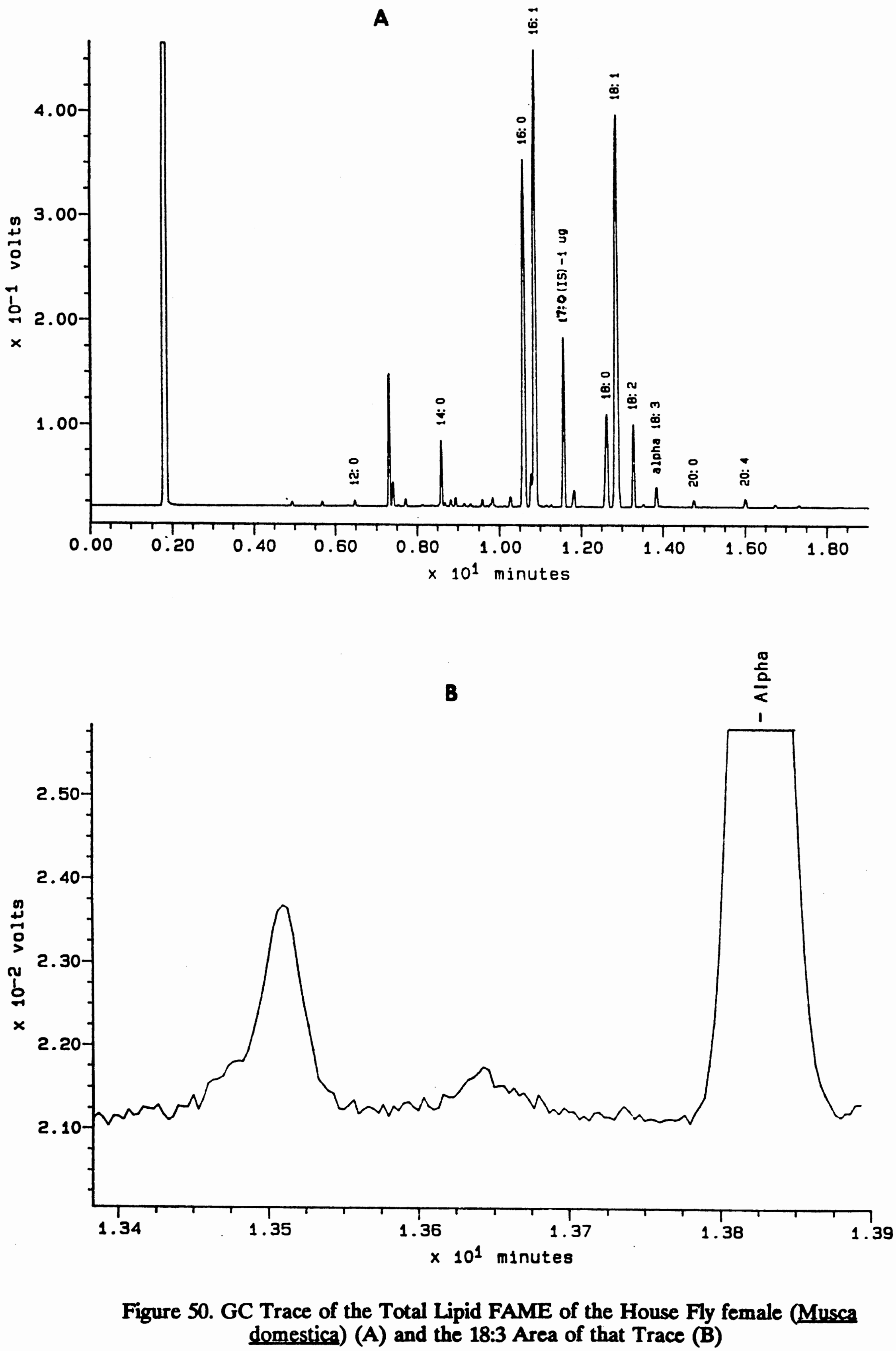
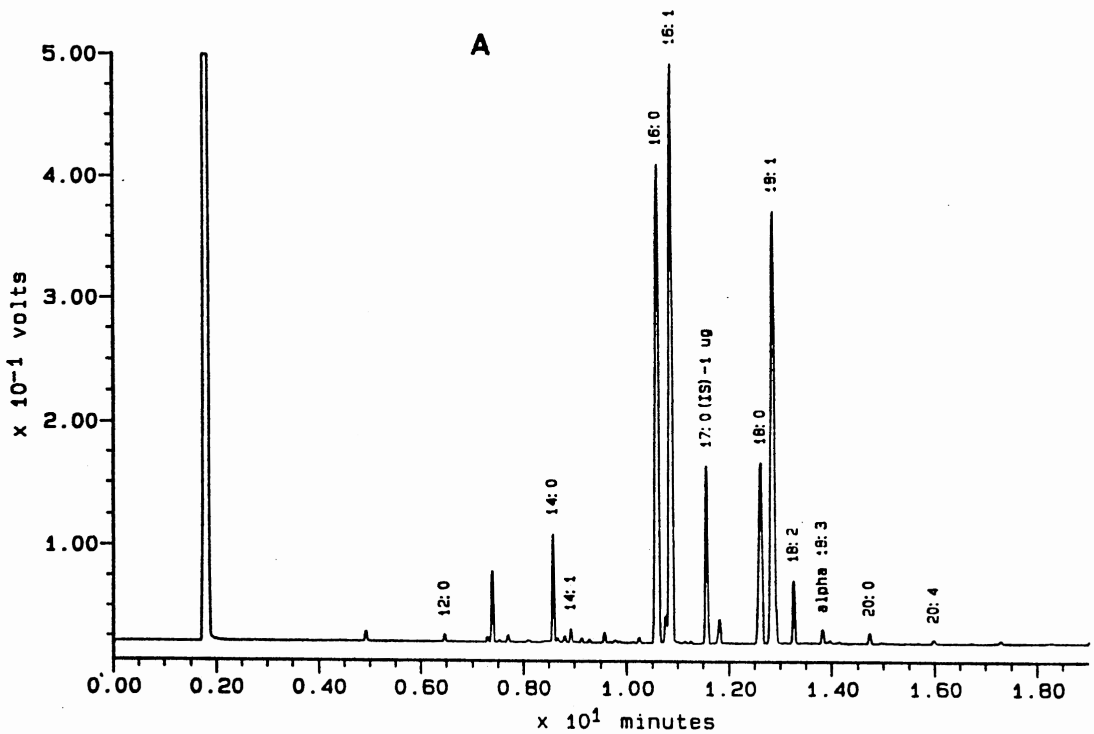


Figure 50. GC Trace of the Total Lipid FAME of the House Fly female (Musca domestica) (A) and the 18:3 Area of that Trace (B)



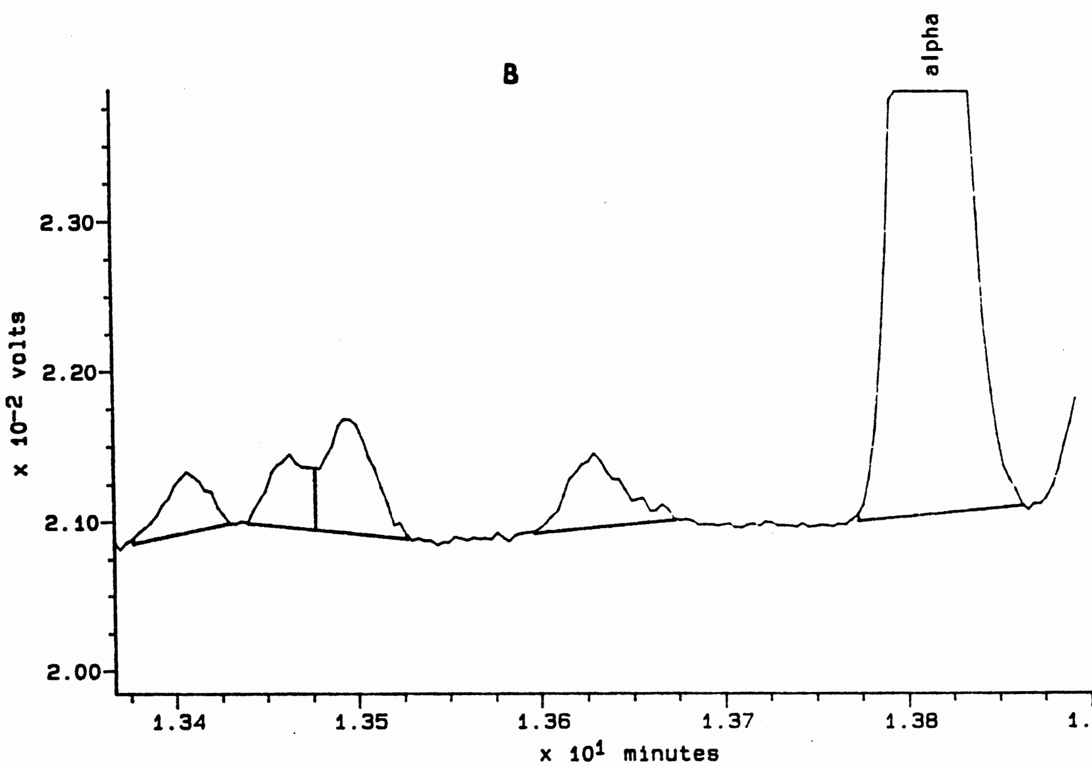


Figure 51. GC Trace of the Total Lipid FAME of the House Fly larvae (Musca domestica) (A) and the 18:3 Area of that Trace (B)

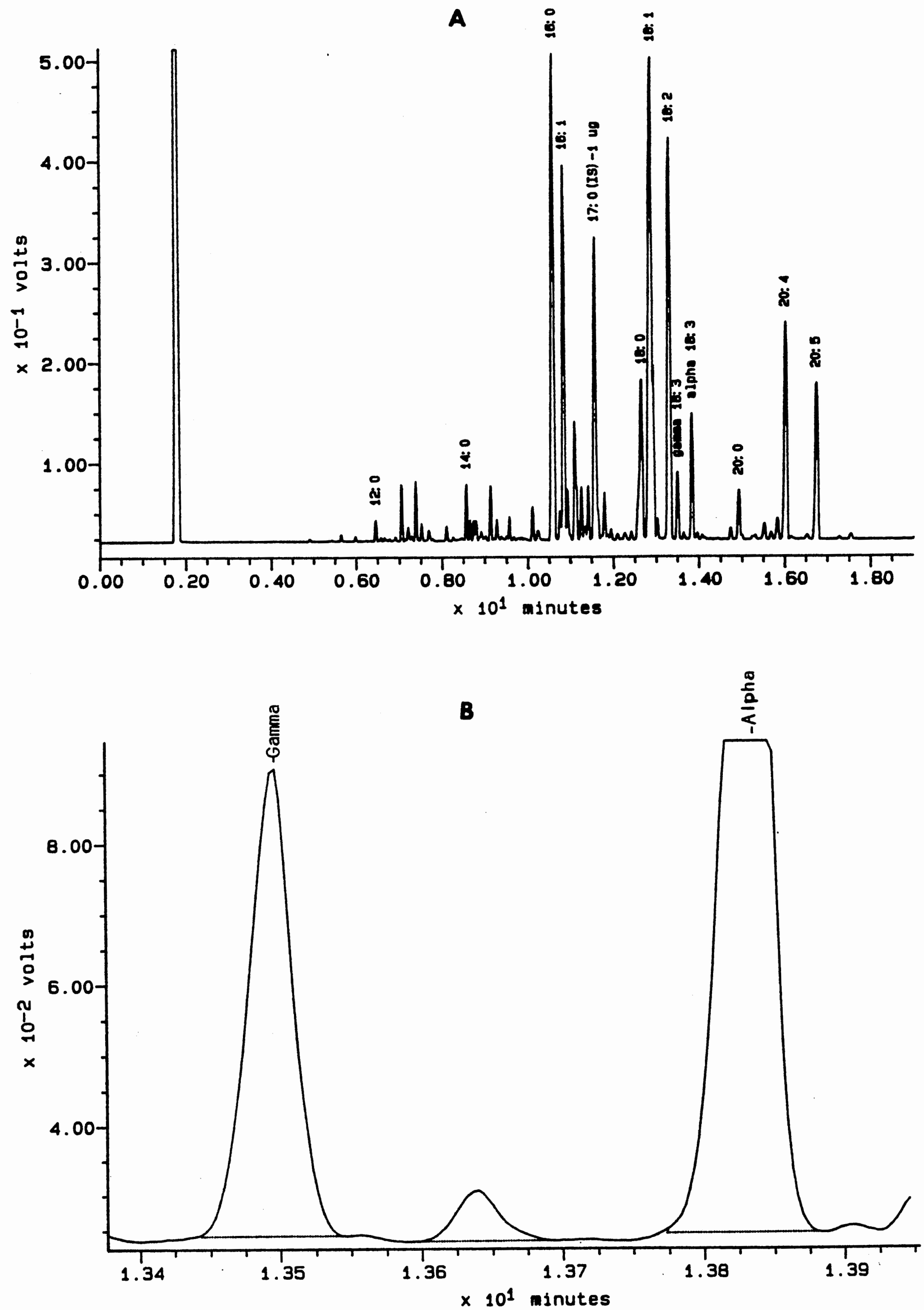


Figure 52. GC Trace of the Total Lipid FAME of the Horse Fly (<u>Tabanus</u> <u>atratus</u>) (A) and the 18:3 Area of that Trace (B)

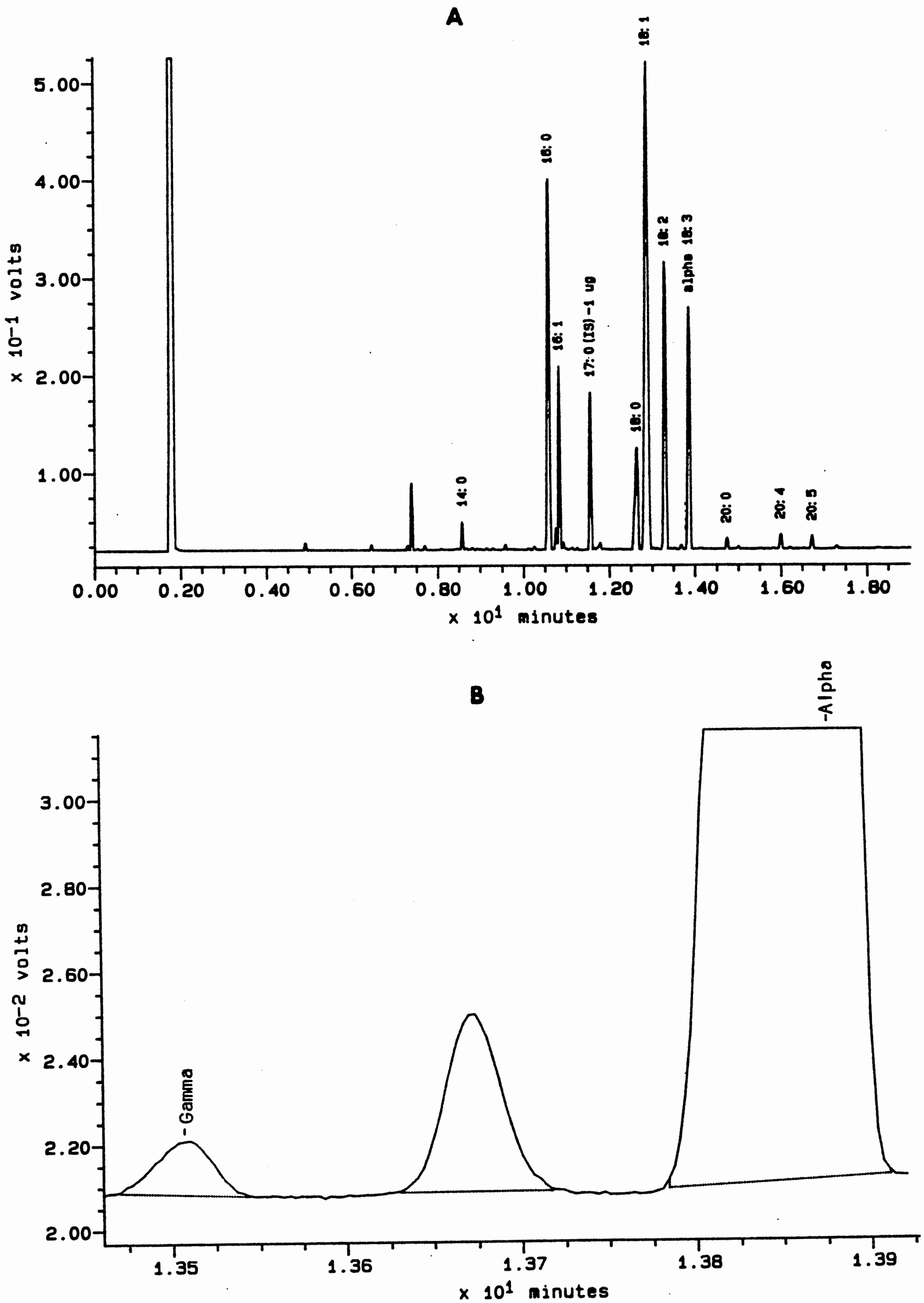


Figure 53. GC Trace of the Total Lipid FAME of the Horse Fly (Tabanus abactor) (A) and the 18:3 Area of that Trace (B)

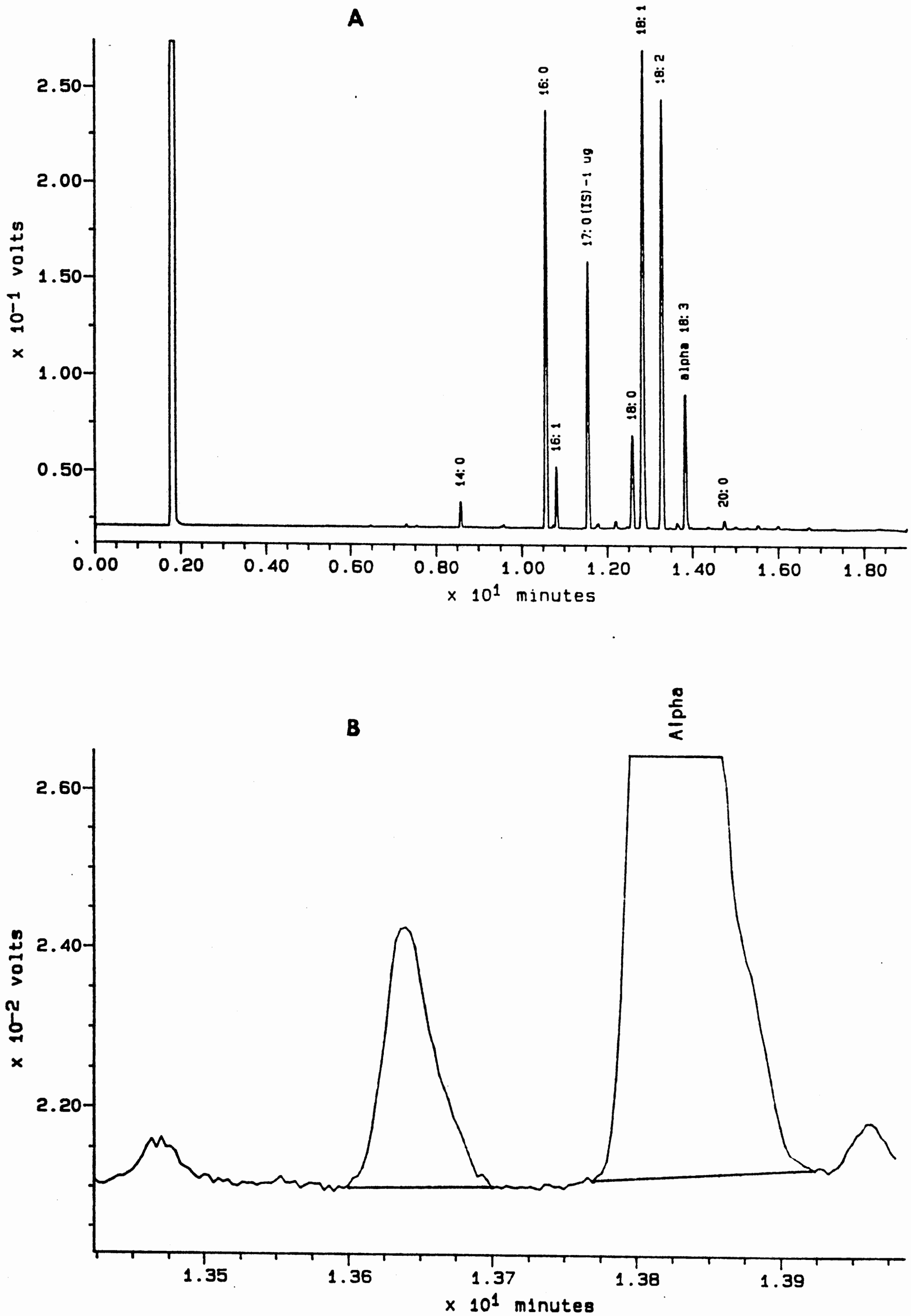


Figure 54. GC Trace of the Total Lipid FAME of the Meadow Grasshopper (Conocephalus f. fasciatus) (A) and the 18:3 Area of that Trace (B)

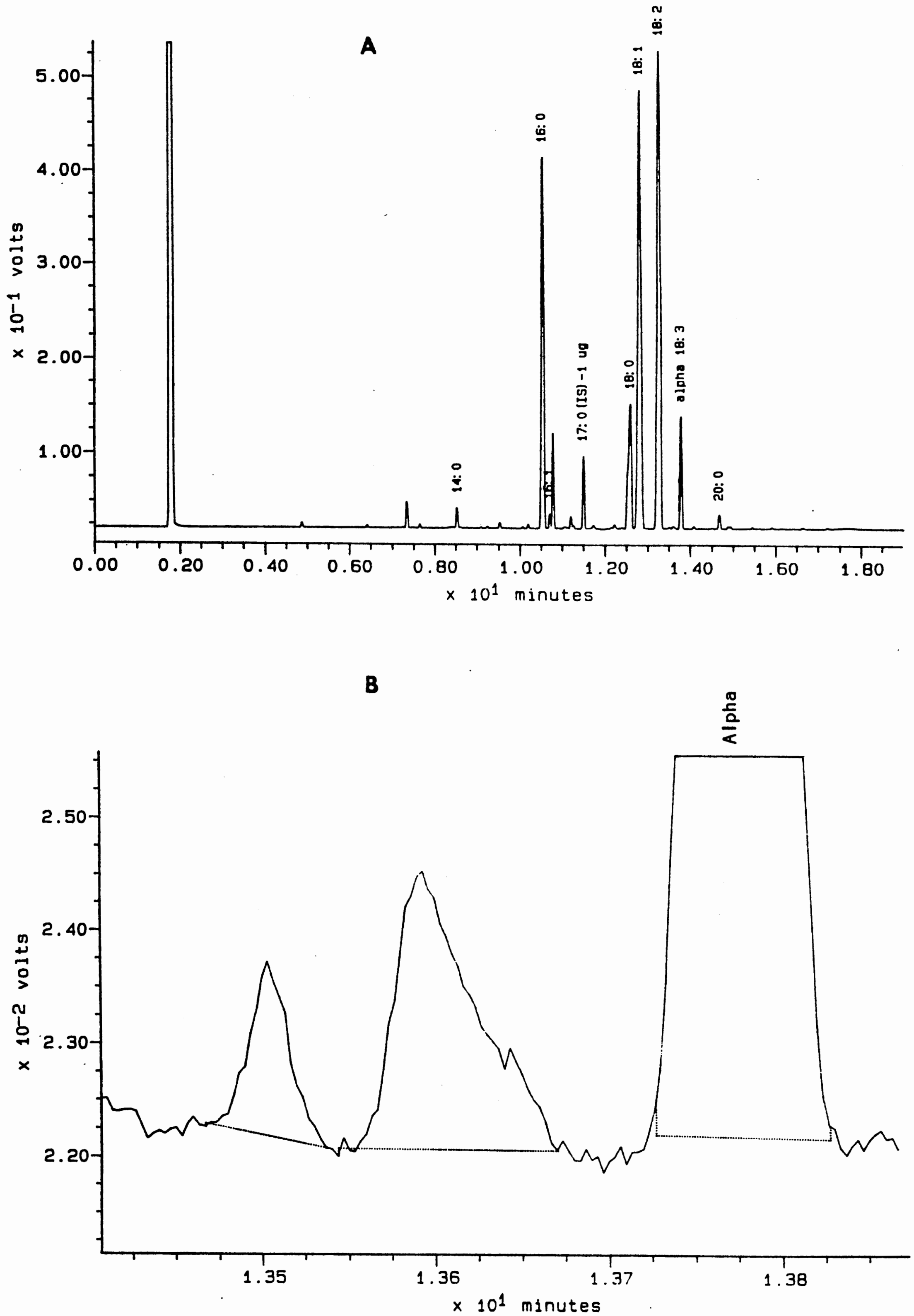


Figure 55. GC Trace of the Total Lipid FAME of the Field Cricket (Gyrllus sp.) (A) and the 18:3 Area of that Trace (B)

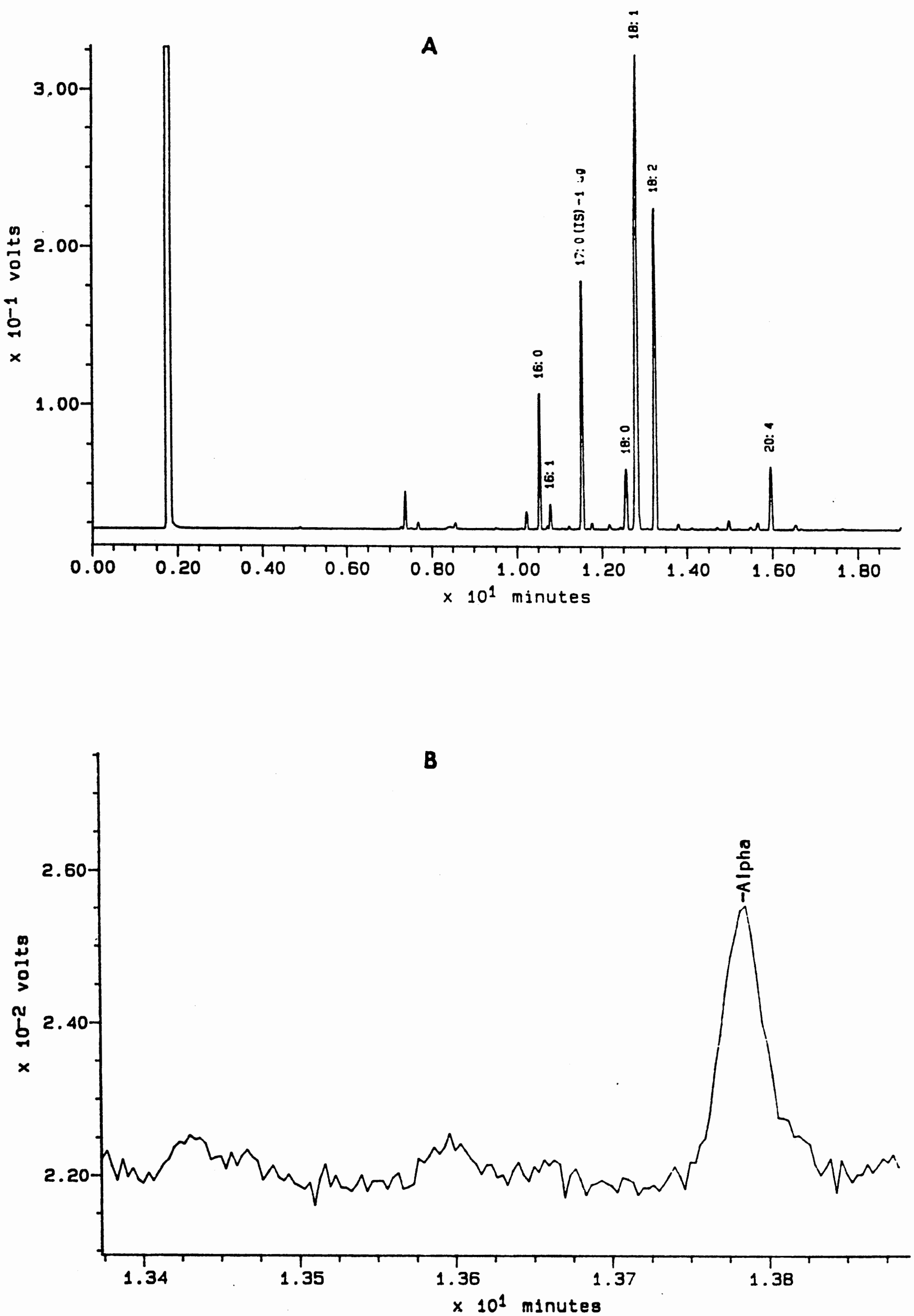


Figure 56. GC Trace of the Total Lipid FAME of the German Cockroach (Blattella germanica) and the 18:3 Area of that Trace (B)

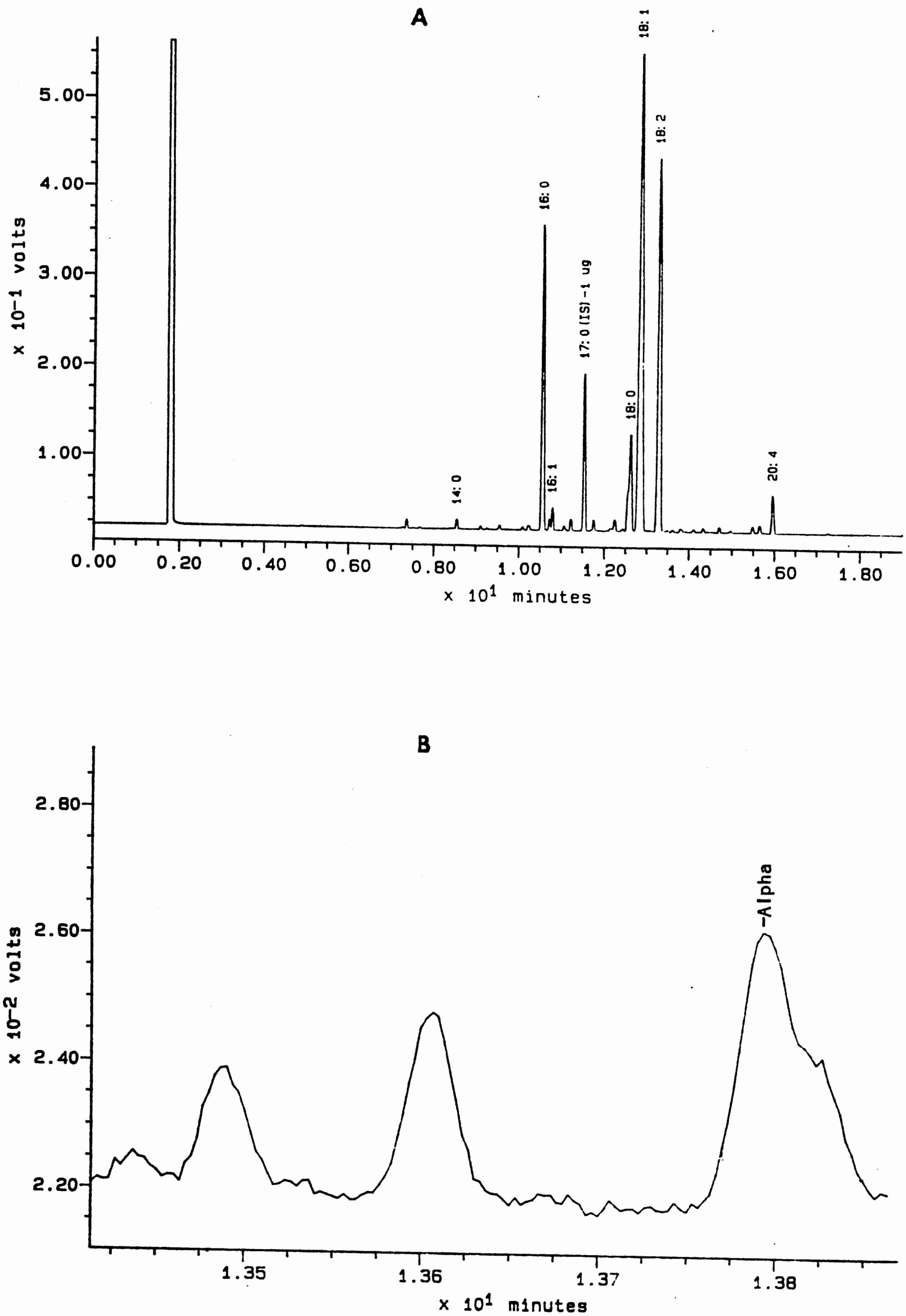


Figure 57. GC Trace of the Total Lipid FAME of the American Cockroach nymph (Periplaneta americana) (A) and the 18:3 Area of that Trace (B)

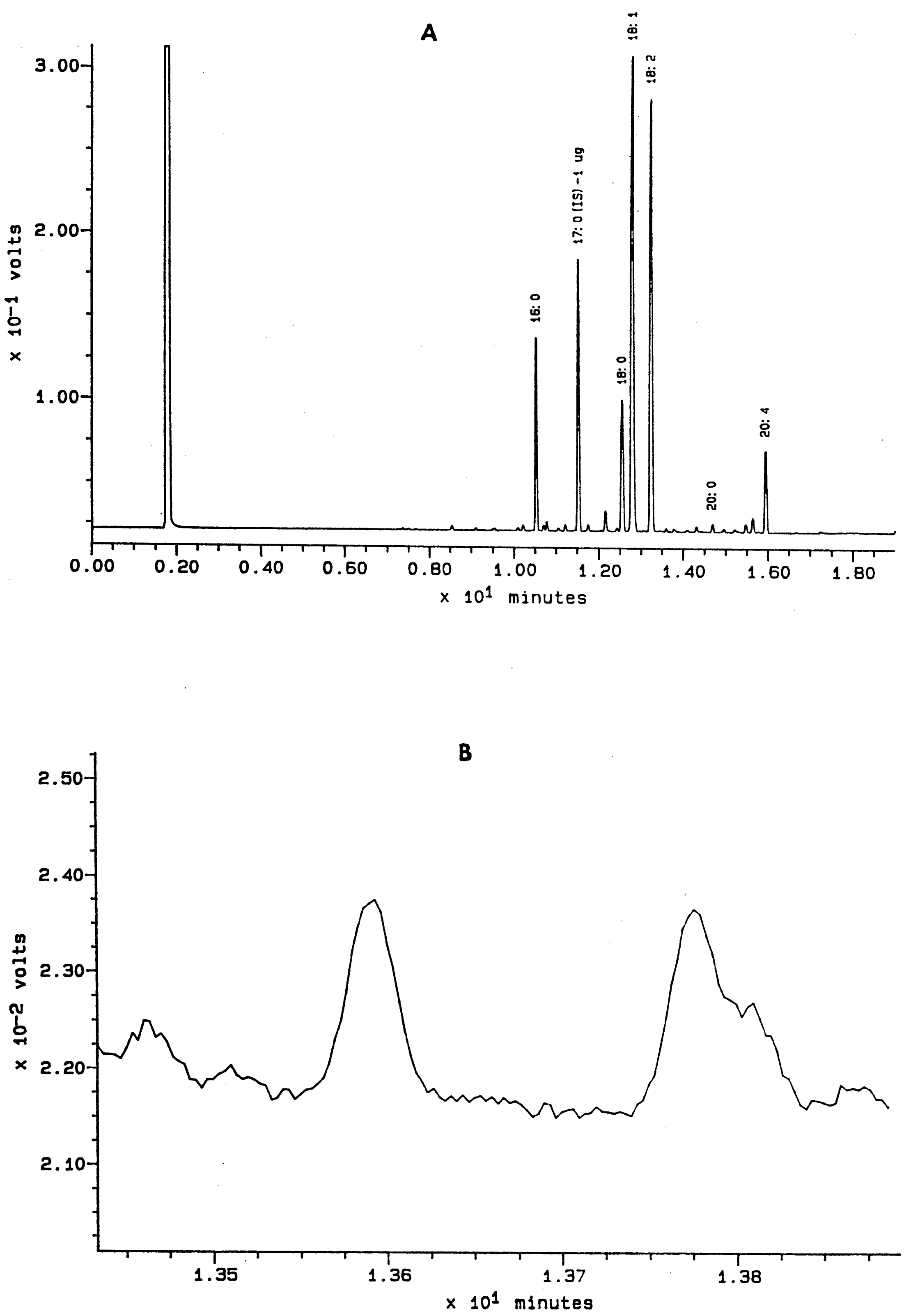


Figure 58. GC Trace of the Total Lipid FAME of the American Cockroach Adult (Periplaneta americana) (A) and the 18:3 Area of that Trace (B)

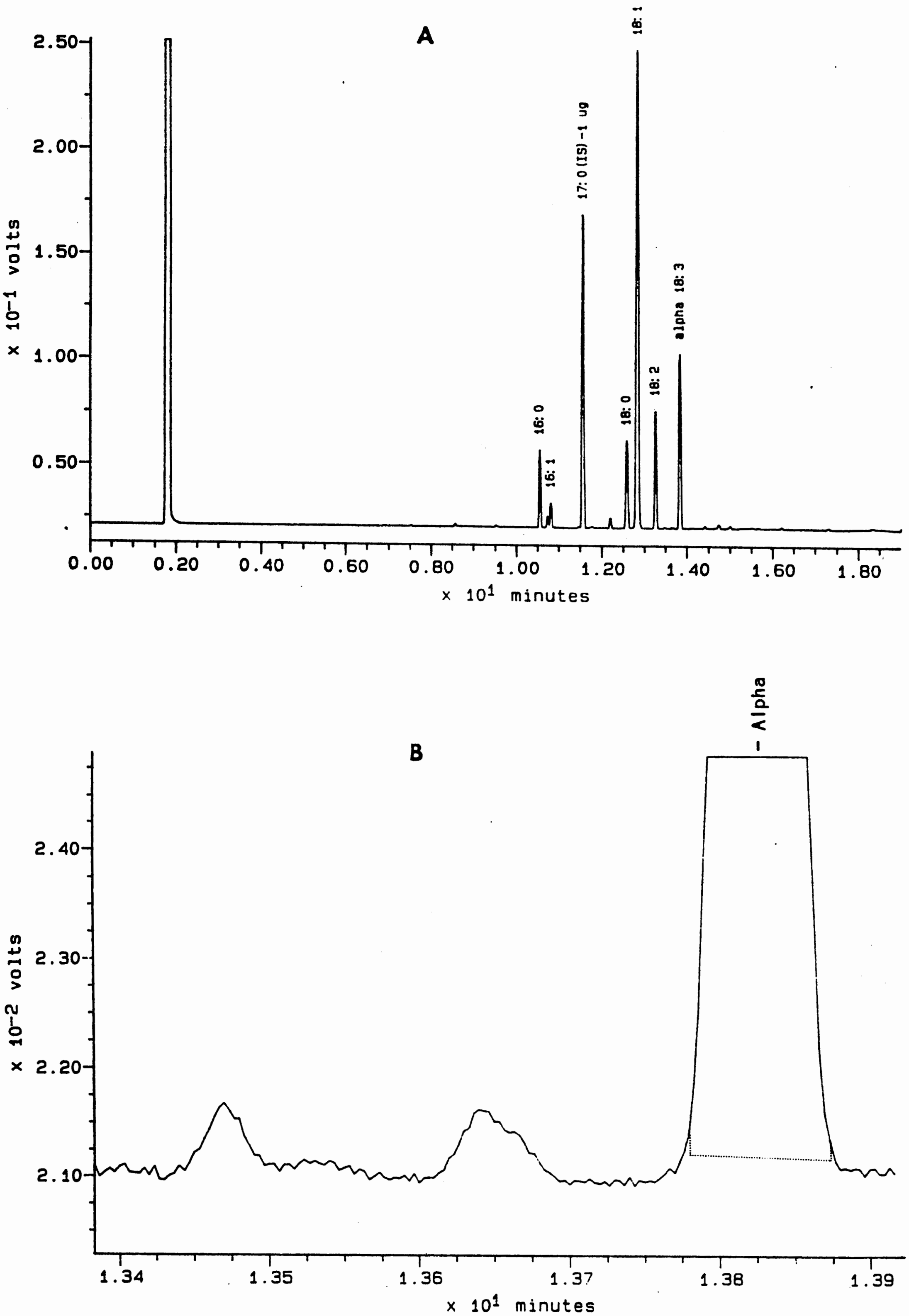


Figure 59. GC Trace of the Total Lipid FAME of the Honey Bee worker (Apis mellifera) (A) and the 18:3 Area of that Trace (B)

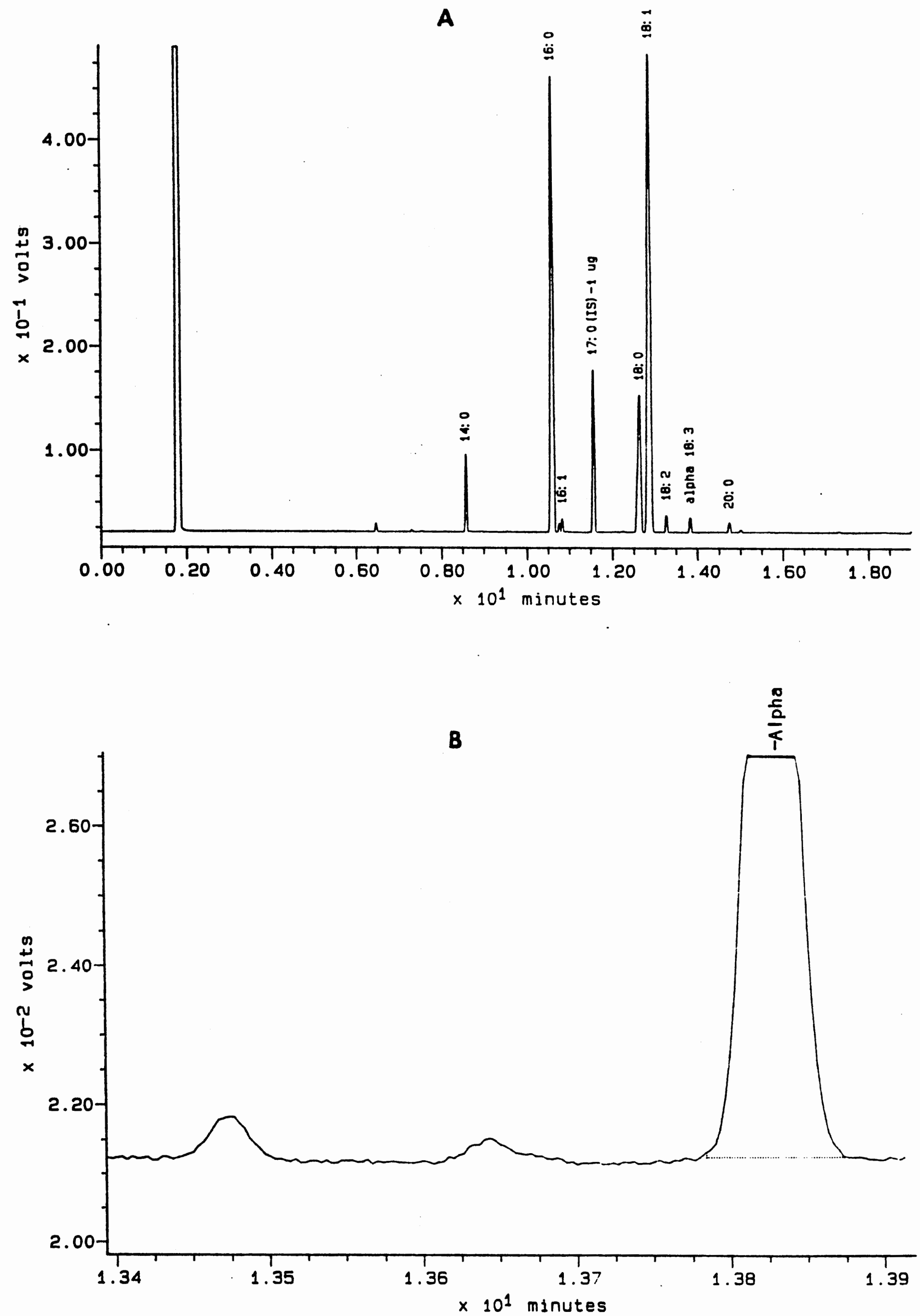
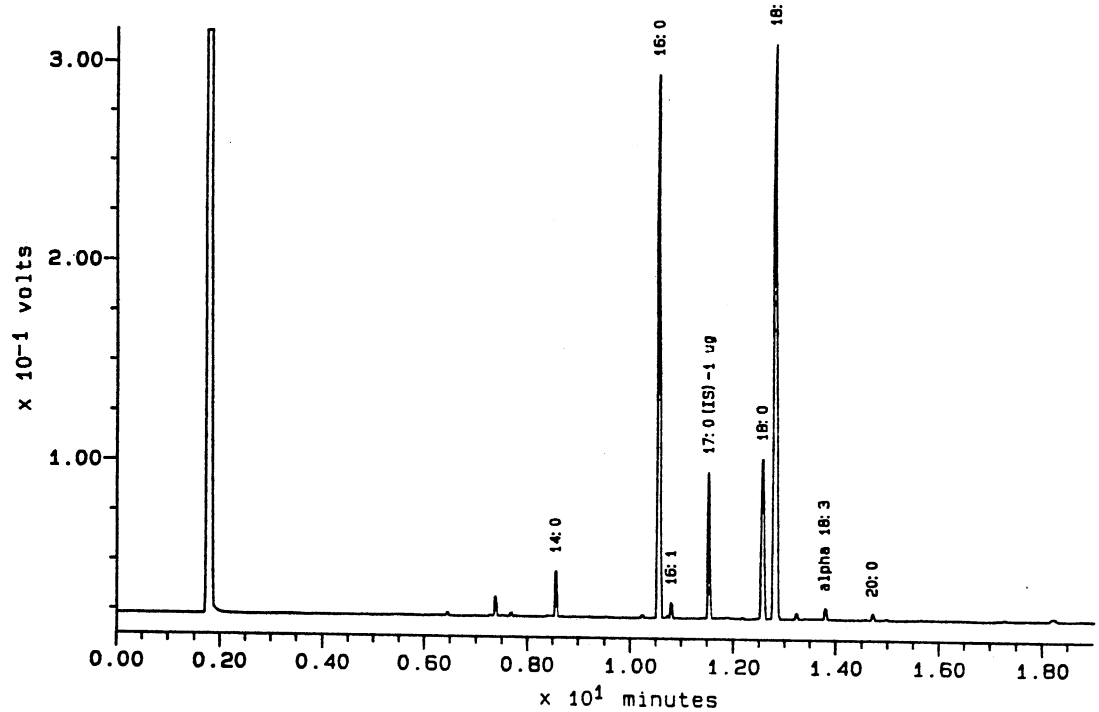


Figure 60. GC Trace of the Total Lipid FAME of the Honey Bee pupae (Apis mellifera) (A) and the 18:3 Area of that Trace (B)

A



B

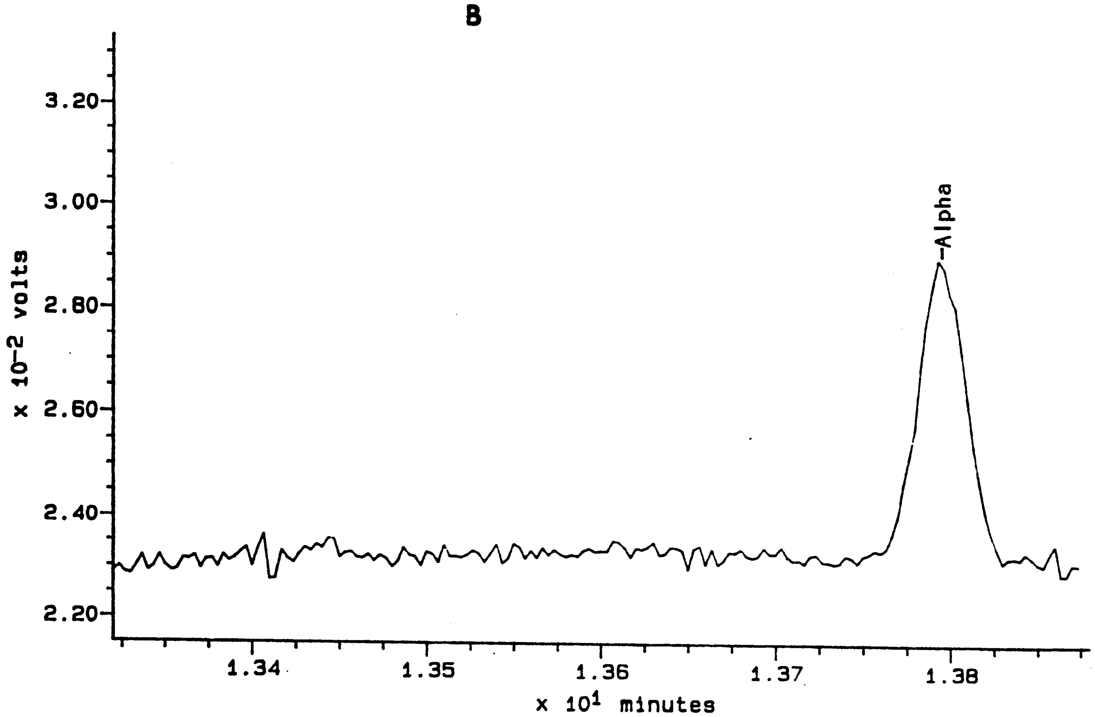
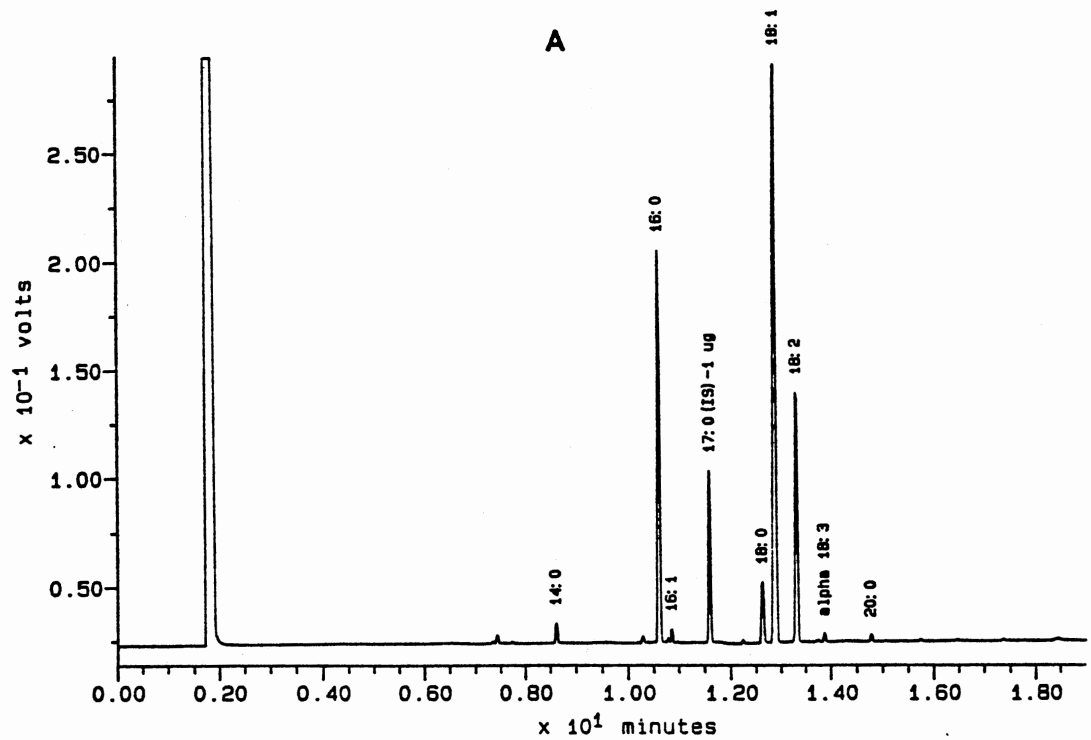


Figure 61. GC Trace of the Total Lipid FAME of the Honey Bee larvae (Apis mellifera) (A) and the 18:3 Area of that Trace (B)



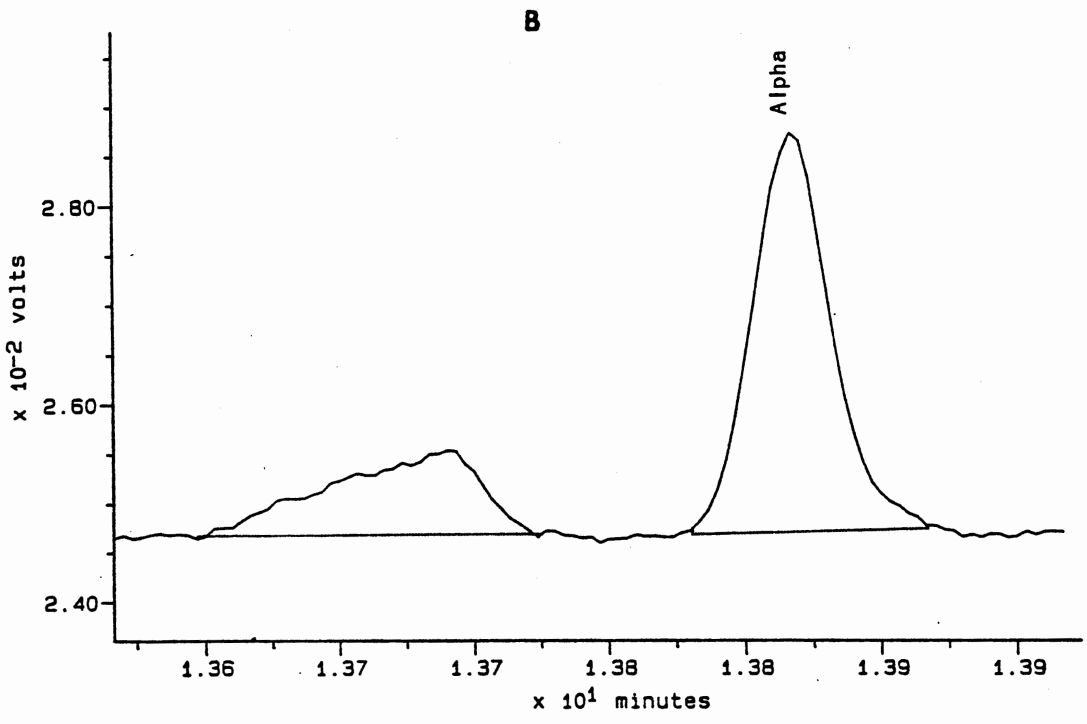


Figure 62. GC Trace of the Total Lipid FAME of the Green Lacewing (<u>Chrysopa</u> sp.) (A) and 18:3 Area of that Trace (B)

VITA

Rose M. Edwards

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Master of Science

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